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The Brandt report: better luck this time?

BLUEPRINTS for global survival have become an increasingly common fixture in popular debate over the past few years. Frequently presented with almost millenarian zeal, their appeal reflects an awareness that the growing interdependence of national economies has also nurtured potentially self-destructive tendencies of global proportions. The report* of the commission, headed by ex-West German prime minister Willy Brandt, suffers, like many of its predecessors, from a heavy overdose of idealism. But it deserves to be taken seriously, because it appears at a time when the stability of East-West and North-South relations is more fragile than for the past twenty years.

In one sense the Brandt Commission — officially known as the Commission in International Development Issues — picks up where the Pearson Commission left off ten years ago. At that time, the main question was the appropriate level of development "aid" which the rich countries should provide to the poor; Pearson's answer was to set a target (which few countries have lived up to) of 0.7% of gross national product. Since then attention has shifted from the level of aid contributions to the conditions under which development can take place. And one way to read the Brandt Commission's report is as a reply to recent vocal demands for a "new international economic order".

The commission's message is that the development of a strong global economy will be of mutual benefit to rich and poor countries alike; but that this will not be achieved merely through the unfettered workings of the global market-place. What is recommended is almost a formula for an international welfare state. Political institutions, the commission argues, need to be developed which will protect the weakest actors on the international scene; bring order to the financial chaos which would result if market-forces were left unchecked; and seek redistribution of global wealth through international taxation.

As a prelude to this strategy, the report repeats a by-now familiar litany of the sad state of the world we live in. Furthermore, many of the specific proposals also carry a familiar ring. Some have already been heard from numerous multilateral aid bodies. Others are more often proposed by the developing countries themselves, and their espousal by a commission calls both for a massive transfer of resources from the developed to the developing nations, and for a strict code of conduct to regulate the activities of multinational corporations. At present both are being resisted, the former most recently at the Delhi meeting of the United Nations Industrial Development Organisation (UNIDO), which ended in stalemate earlier this month. There are other, less familiar, suggestions. For example, the commission devotes a chapter of its report on energy. It recommends that there should be a global energy research centre, established under UN auspices.

But it is in the field of economic reform that the commission makes some of its most far-reaching, controversial, proposals. Here the commission is calling for no less than the restructuring of international financing mechanisms, particularly to cope with the cash surpluses arising from the oil revenues of the OPEC countries. One of the most significant aspects of the redevelopment scene over the past decade, it points out, has been a shift from government-financed aid to loans from private banking and commercial institutions, many of which have used such loans as a way of recycling OPEC deposits.

The commission argues that this massive recycling has created instabilities in the system which the private sector may prove inadequate to control; and that the massive debt burden already carried by many Third World countries will inevitably dominate

development efforts for many years to come. Furthermore aid offered on commercial loan terms will tend to encourage relatively short-term development needs. But the commission is also critical of the stringent conditions laid down by the International Monetary Fund for loan assistance, arguing that these have often "tended to impose unnecessary and unacceptable political burdens on the poorest", and have led countries to prefer the private international banks.

The solution suggested by the Brandt Commission is to institutionalise ways of liberalising the international financing system. With respect to the IMF, for example, it suggests that the fund should "avoid inappropriate or excessive regulation" of the economies of the developing countries, and not impose "highly deflationary measures as standard adjustment policies". As far as the World Bank is concerned, the commission suggests, among other things, a shift to greater emphasis on programme loans, which would give a country flexibility in the methods it adopts to achieve a certain broad objective; it also suggests decentralising the bank's activities from Washington and giving a greater role in decision-making to representatives of the Third World.

Talking in realistic terms, any moves which would circumvent the role of existing financial institutions are certain to meet considerable opposition. Equally controversial is the suggestion for an international taxation system, all countries being assessed for their ability to pay into a general fund which would be made available for development purposes. The political appeal of such a system, possibly taking the form of a tax on international trade, is obviously limited; yet in his introduction to the report, Willy Brandt states that "by the end of the century the world will probably not be able to function without some practicable form of international taxation."

Those who followed last year's UNCSTD debate closely will not have missed the irony. For one of the major stumbling blocks in Vienna was the refusal of the developed countries to agree to the creation of a new fund, proposed by the developing countries to be based on contributions automatically assessed as a percentage of the trade in manufactured goods between rich and poor countries. It was merely agreed to establish an interim fund, based on voluntary contributions.

Indeed the commission's sketchy reference to the UNCSTD conference, grasping the inadequacy of its conclusions but providing little insight into why no more was achieved, merely illustrates the report's major weakness. For it is written more as a political manifesto than as a realistic framework for planning; but one that, given the growing attractions of protectionism and monetarist policies, lacks wide appeal at present. Furthermore the message that it is in the self-interest of the rich to promote the development of the poor inevitably invites the scepticism of those who would claim it as an expression of neo-imperialism.

But in one instance at least, the commission is correct. Suggesting that the industrialised countries restimulate their efforts to meet the aid targets to which many are already committed — and indeed to aim for 1% of GNP by the year 2000 — it adds that "what the neglect of foreign aid expresses is ultimately the lack of political priority attached to it." Next month in New York will see the pledging conference for the interim fund recommended by UNCSTD to finance new scientific and technological endeavours in developing countries, administered by the United Nations Development Programme. The event will indicate how much the main recommendations of the Brandt Commission have been taken to heart. Current indications are that total contributions will be less than initially hoped for although some countries — Britain excluded — will feel it appropriate to make reality of the Brandt Commission's analysis; and the acceptability of its message. □

*"North-South: A Programme for Survival" the Report of the Independent Commission on International Development Issues. Published by Pan Books, London, £1.95.



High energy physics

Money may determine West Germany's view

IN the battle — imagined or otherwise — between the Hamburg laboratory DESY and the European subnuclear physics centre CERN near Geneva, Germany will give CERN priority, unless outside factors intervene. So Dr Günther Lehr, director for international cooperation of the German federal ministry for research and technology, told *Nature* last week.

The pace of diplomacy over the issue between Italy and Germany is increasing. Italy was the first to raise doubts about German intentions. In the past few days the Italian minister of science, Vito Scalia, met Lehr with the chairman of the European Committee for Future Accelerators, Professor Marcel Vivargent, in Zurich — construed as neutral territory. And Professor Herwig Schopper, director of DESY and holder of all but Italy's vote to be CERN's next director-general, visited Scalia in Rome to clarify his position.

However, according to a Rome diplomat "it is still too early to say" if all the problems are solved. "Time has been useful" he said. "It has removed the cloud to a certain extent, there has been a clarification of problems."

Italy, which since the closure for high energy physics of its ADONE electron ring at Frascati near Rome has relied heavily on the CERN facilities at Geneva, fears competition between DESY's plans for an electron-proton ring, HERA, and CERN's plans for a large electron-positron ring LEP. The first stage of HERA, which would probably operate some years before LEP, would also be an electron-positron ring though at a nominal energy of 35 GeV on 35 GeV (see *Nature* 31 January page 420), below reach of the intermediate vector boson. Italy has perceived Schopper's potential appointment to the CERN director-generalship as a "Trojan horse" which would lead to the running down of CERN and the aggrandisement of the DESY laboratory in Hamburg.

But Lehr dismisses these fears. "DESY has risen in the last few years from a purely national laboratory to one of international importance" he said last week. "At first this caused surprise, and now there is over-reaction."

Lehr is now convening a small group of scientific advisors to make recommendations, sometime this year, on spending in Germany on ten 'big projects', including LEP and HERA. Others include a new neutron source, equivalent to the UK Rutherford Laboratory's spallation neutron source now under construction, a European synchrotron radiation source as proposed by the European Science Foundation, improvement of the heavy ion accelerator at Darmstadt, and a deep drilling project to reach the Mohorovicic

discontinuity, the 'Mohole' project dropped by the US some years ago.

Since Germany contributes 25% of CERN costs (the maximum allowed), the costs to it of LEP and HERA are roughly equal. But, said Lehr, "I would assure Scalia in Zurich that the LEP and HERA decisions would be independent. I cannot see us doing HERA instead of LEP. I rule out doing neither, but I see no basis for choosing HERA in favour of LEP, unless it is for reasons outside our control."

Such reasons might be objections to LEP from other member states of CERN. The Scandinavians particularly are thought to be cool to LEP (after all Hamburg is closer than Geneva) and the UK is likely to be in favour only if LEP is built slowly, to reduce annual costs.

However, cost is also a prime consideration in Germany, and Lehr does not know how much money will be available to him. Discussions are presently taking place on the science ministry budget for 1981, and it appears that there will be only "a relatively small increase" constrained by the international economic and political situation. There will not be enough money for all the big projects, but may be enough for more than one — perhaps 50 million DM per year.

"In these circumstances", says Lehr, "I cannot forecast my minister's reaction" to a proposal to go ahead with LEP and HERA together. This uncertainty leads directly to Italy's doubts. If Germany decided to go ahead with HERA before there is a decision on LEP (which will not come before the CERN council meeting of June 1981 at the earliest) would Germany be inclined to temper its enthusiasm for LEP?

Lehr regretted the Italian position on Germany and LEP "but in some respects I understand them, because DESY has been successful. CERN — I choose my words carefully — has been better at building superb accelerators than at discovering

spectacular physics."

Why should this be so? Because, suggested Lehr, the director general of such international bodies tries always to be on the safe side, to build an accelerator which runs on the touch of a button. "We should consider this matter" said Lehr, who is also vice-president of CERN Council, "and perhaps change this policy. We could build this smaller, cheaper, and take risks . . . It will not be possible to build larger and larger particle accelerators: governments and other scientists won't accept it." Schopper, Lehr believes, would be a director general who would take imaginative risk, as he has done with DESY.

The Italian science ministry, however, told *Nature* last week that there could be no decision on the director generalship, Rome's main political weapon, before the June meeting of CERN Council, and that the time before then should be used to clarify the issues further. Lehr, on the other hand, insists that Schopper's early appointment as director-designate — the post is effective from January 1981 — would help to clear the ground for LEP. Strictly, a two-thirds majority is all that is required for a decision, but it is felt that unanimity is necessary on this appointment.

Scalia and Lehr appear to agree on one thing: the need for speed with LEP, if it is built. Lehr would like it built in five years rather than the seven which is current CERN thinking. This can be done within a 'constant' CERN budget of 620 million SwFr a year, says Lehr, if a minimum one-sixth version of LEP (with a maximum luminosity at 49 GeV) is adopted. Italy is in agreement with this. It would entail the same tunnel, but having fewer accelerating cavities so that the machine just reaches the mass of the intermediate vector boson, the Z_0 .

Lehr sees US competition for the Z_0 as less of a threat than some: "John Deutsch has told me that his department of energy will spend some \$300 million on Brookhaven National Laboratory, Fermilab, and the energy doubler, and Richter's Z_0 factory, so you can expect delays for lack of funds."

Rome does not regret the stand it has taken. A diplomat in the science ministry told *Nature* last week "our pressure has produced an increased awareness of the need for speed. The matter has been brought into the limelight." The diplomat "found room for agreement" in the language now used by the German delegates to the CERN Council. "We believe Germany is a very strong member of CERN, and wish it to remain so."

Robert Walgate



Schopper: CERN's next director-general?

United States

USDA to study potential anti-social effects of sponsored research

FOLLOWING the US Department of Agriculture's decision to stop funding research that would throw farm workers out of their jobs (*Nature* 3 January 1980 page 5), Agriculture Secretary, Mr Bob Bergland, has now announced a study into the possible anti-social effects of all federally-funded research.

The task force that will undertake the study will concentrate on the \$1 million which the Department spends annually on research at universities and land grant colleges into the mechanisation of agricultural production. This has been subject to criticism that led to the decision to halt new funds for such research.

In a speech to the staff at the Department's Science and Education Administration at Reston, Virginia, Mr Bergland repeated that he would no longer support research whose benefits would be restricted to a particular locality or social

group, or which posed a threat to the natural environment or social community.

"In the specific case of mechanisation research, this means that we will not put federal money into research where — all other factors being equal or neutral — the major effect of that research will be replacing an adequate and willing workforce with machines", Mr Bergland said.

However he added that the economic incentives of the market-place should be powerful enough to enable this type of research to be funded by private industry, either by itself or with the help of state funds.

Mr Bergland said that this policy was consistent with the department's responsibilities to meet national needs, rather than those of a particular group. The government should not finance research whose beneficiaries could, in an extreme case, use it "to gain control of the farm-to-

market structure, monopolise the sources of finance at every step, and increase their profits by selling what may well be an inferior product at a price that is insulated from competition".

He pointed out that until recently, agricultural research had been evaluated almost exclusively in terms of what the research promised in the way of volume productivity gains; too often potentially significant negative economic and social effects were not factored into the evaluation.

"The result was a skewed cost-efficiency statistic and distorted perspective. If this is not remedied in the research evaluation process, it will surely handicap our ability to meet changing national priorities", Mr Bergland said. This was the reason for establishing a task force to look for research projects involving "the dubious use of federal money." □

New group to study cryptography policy

WAYS of resolving conflicts between the academic freedom of researchers working on computer codes and the interests of national security are to be investigated by a group that has been established by the American Council of Education, under a grant of \$32,000 from the National Science Foundation.

The chairman of the study group will be Dr Werner Baum, dean of the college of arts and sciences at Florida State University. Dr Baum was previously chancellor of the University of Wisconsin at Milwaukee, where in 1978 a computer scientist, Dr George Davida, had a patent application for a new computer code blocked by the Department of Commerce following objections from the National Security Agency.

Dr Baum told *Nature* recently that he hoped the study group would be able to recommend policies and procedures which would both protect the legitimate academic and property concerns of universities and research institutions on the one hand, and the concerns of the US government about security on the other.

He added that this might involve changes in the law. In the Davida case, for example, which was successfully challenged by the university, Dr Baum pointed out that the NSA had no due process requirement covering demands that an individual should not publish the results of his research for national security reasons. Current law, he said, which goes back to

the post-war McCarthy period, may turn out to be non-constitutional.

The ACE working group was established after NSA director Admiral Bobby Inman last year suggested a dialogue with the scientific community on conflicts between academic freedom and security issues. The group will include representatives of professional societies such as the American Mathematical Society and the Computer Society of the Institute of Electrical and Electronic Engineers, as well as legal advisers. It is expected to produce a report within a year.

Last year Admiral Inman was quoted as supporting legislation which would control research and the subsequent publication of results of "a central core of critical cryptologic information . . . that is likely to have a discernable adverse impact on national security." □

Battelle paints healthy picture of US R&D

IN contrast to the frequent complaints still heard about the health of US research and development, a surprisingly lively picture of the current situation is presented in the latest annual analysis by the Columbus Laboratories of the Battelle Memorial Institute. Taking R&D expenditures across the board, Battelle estimates that these will grow by 19.7% in 1980, to a total of \$61.8 billion. Within this total, industrial funding is expected to grow faster (20.9% increase) than federal funding (19% increase); but even given a predicted cost increase of 12%, this still implies a 7% real

growth in expenditure, reaching a new peak in total real funding of R&D, the report says.

Despite the unfavourable economic climate, the Battelle report says that there are many intangibles, "particularly the reawakened feeling on the parts of many decision makers that R&D is essential to our long-term economic health — that favour greater-than-usual increase in support for research. The report says that its analysis indicates that the upward movement established over much of the past decade in R&D expenditures is likely to continue, with the 1980 figures leading to an average increase over the past seven years of 3.3% in real terms. It estimates that during the decade of the 1980s real R&D activity will increase at an average rate of approximately 3%.

The major sector to indicate increases in both funding and performance of R&D in 1980 is expected to be industry. In contrast, academic and other non-profit institutions are expected to register small decreases in the percentage of support and performance (3.5% and 15.1% respectively); but there will still be significant increases in the total activity of each (1.3% and 17.6% over the estimated 1979 figures).

The report says that therefore in spite of inflation and the desire of the Carter administration to balance the budget, there are signs of greater support for research. It points out in particular that industrial support is growing in fields affected by regulations and in those fields most directly influenced by the need for more energy-efficient products and processes. □

Finland

Public confidence in science is reviving

LAST week, Finland's Swedish-language technological society, the Tekniska Förenigen 1 Finland (TF1F) held a panel discussion on the country's energy policy as part of the centenary celebrations of the society's foundation. According to Erik Moring, the president, of TF1F, the holding of this discussion, between five of the country's leading experts from both the government and private sectors, reflect the general theme of the year's celebrations — to show the country that scientists and technologists are not "some sort of monster."

The TF1F celebrations, with their strong emphasis on the best use of Finland's natural resources for the good of the country as a whole, coincide with Finland's current "environmental year", and a general swing of public opinion away from the strongly anti-science bias of the mid and late 1970s. On 8 February, the Social Democrats (the largest single party in the Finnish parliament) called for a major investment in science and the creation of 300 new research posts. A few weeks previously, Finland's highly charismatic president, Urho Kekkonen, called for a reassessment of R&D investment and the expansion of research facilities — the first time that the president called so directly for a boosting of science since 1964.

The result that previous appeal was a major reorganisation of research planning, leading in 1969, to the foundation of the Academy of Finland, which, in spite of its name, is essentially a system of research councils and of government science funding. When the Academy put forward its five proposals for increased government science funding as "areas of emphasis", these had a strong bias towards the social sciences: environment, work and working conditions, public health, basic necessities of the population and living conditions, and the implementation of democracy and equality. It was this last project that, in the opinion of many Finnish scholars, led to the breakdown of public confidence in science planning.

Undoubtedly the project did provide a vocal focus for grievances. According to its opponents, the results of the research were widely politicised by the left. A nationwide debate sprang up in the press causing considerable disillusion among the public. The academic professions, which increasingly tended to range themselves against the Academy, had, however, other complaints.

During the past 25 years, Finland's general policy of decentralisation has increased the number of the country's universities from two (Helsinki and Turku)

to 18 (the last of which, the university of Lapland, was founded in 1979). Some of these are genuine new foundations, others are upgraded/former specialist or teacher-training colleges (e.g. the University of Jyväskylä). This proliferation was, to a large extent, necessary to prevent a permanent brain-drain to the south-west of the country.

However the expansion has left Finland, like many industrialised countries, with the problem of a very slow turnover of research staff in universities and few opportunities for new graduates who wish to stay within the Finnish academic structure.

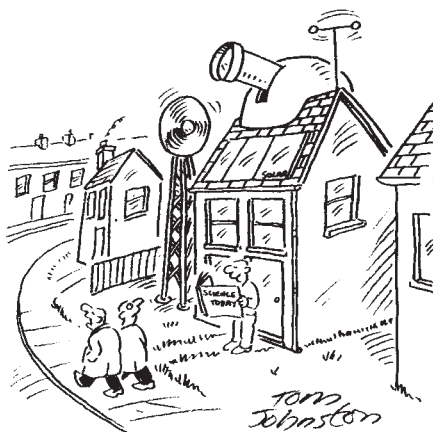
An even more bitter cause for resentment, however, was the government regulation of the 1960s codifying and standardising the granting of academic degrees. This was, not surprisingly, seen as an encroachment on academic liberty, particularly since Finnish tradition followed the 19th century German ideal of

This laboratory was established by Professor Olli Lounasmaa in 1975 on the principal that low temperature physics is one of the frontier areas still within range of the Finnish budget. At present the laboratory holds the record of 50 nano-Kelvins (using a copper nuclear spin system). One byproduct of this work is the development of SQUIDS (superconducting quantum interference devices) to produce magnetic shielding. A magnetically shielded room is now under construction and an international seminar is to be held in May for potential users of what Professor Lounasmaa describes as "this rather expensive facility which will incidentally, be paralleled only by one under construction in Berlin."

Professor Lounasmaa's expertise in planning the laboratory has led to his being appointed head of a new working group to advise the government on the funding of basic research. Finland, he told *Nature*, has a very small bureaucracy. A scientist can therefore hope to obtain funds without too much red tape. Also, although the country has no long tradition in science, the general public, he says, has on the whole a high esteem for science. The disillusion of the last few years is not, he feels, significant in the long run.

Certainly one can find numerous examples in Finland of public confidence in science. Last autumn, the leading Finnish daily, *Helsingin Sanomat* celebrated its ninetieth anniversary with a special series of articles on science — which, in the opinion of Dr Elizabeth Helander, research director of the Academy of Finland, is the principal cause of the recent public change of heart. Some such gestures have involved considerable sums of money. In 1967, to mark the golden jubilee of the country's independence, the Bank of Finland set aside securities worth 300 million FM (\$45 million) whose interest would be used to fund loans for R&D expenditure in the private sector. Some considerable financial daring is also involved in two new publishing projects. One, proposed by the Academy of Finland, is the publication of a new popular science journal which is expected to become financially independent within three years. The other is the publication of a five volume full colour encyclopaedia of Finnish wild life (the first ever such work), which is expected to sell 10,000 at 1600 FM (\$220) the set. In a country of 4.5 million inhabitants and with virtually zero prospects of foreign sales, these figures assume that one household in ten throughout the nation contains at least one science enthusiast.

Vera Rich



"He's our local science enthusiast!"

university autonomy. One result was the establishment of the independent 'Foundation for Research in Higher Education and Policy', an organisation at present smarting under the latest OECD report which described it as a protest group of outraged academics. This, its spokesman, Dr Juha Vuorinen told *Nature*, is simply no longer true. "We are now cooperating much more (with the government) and the political issues are not so hot. What we are trying to do now is to influence the government to give more money to research. Research must be led by experts, not bureaucrats".

Certainly there are fields in which Finland is capable of taking a lead position. One is nitrogen fixation, traditional since the time of Professor Virtanen, who received the Nobel Prize in 1945. Another is the ultra-cold laboratory of the Helsinki University of Technology at Otaniemi.

NEWS IN BRIEF

East-West "persecution" row at Hamburg Forum

USSR Academician Nickolai Blokhin has accused the US and UK of "trying to make a 180 degree turn" in discussing international scientific cooperation and "interfering in the internal affairs of the Soviet Union". Speaking at the Hamburg Scientific Forum this week, Blokhin, head of the Soviet delegation and president of the Academy of Medical Sciences of the USSR, accused them of "trying to be teachers of other countries when no-one asked them to". He was reacting to strong appeals from Lord Todd, head of the UK delegation and president of the Royal Society, and Dr Philip Handler, president of the National Academy of Sciences, on behalf of persecuted colleagues. Lord Todd cited the Soviet Union's behaviour in blocking invitations to scientists to go abroad, and the harsh treatment meted out to scientists "for comparatively minor disagreement with authority".

"Does the Soviet government yet realise", he said "the resentment this causes amongst young scientists? I call on our Soviet colleagues to impress on their government the urgent need for change".

Dr Handler stressed that many Soviet scientists were victimised owing to their wish to monitor the Helsinki Accords. There had been, he said "a spontaneous upswelling of reaction in the US." His delegation was present in Hamburg in spite of many high level scientists' appeal for a boycott.

Blokhin did not answer the charges specifically, but harangued the Forum with a long list of Soviet scientific achievements which he said the US and UK were trying to deny. It had been expected the Soviet delegation would be led not by him but Dzherman Gvishiani, deputy head of the State Committee for Science and Technology.

US probes illicit Soviet technology sales

THE US Department of Commerce is currently investigating numerous cases of illicit sales of computer, laser and transistor technology to the Soviet Union, according to a document leaked to the *Los Angeles Times*. Although black market sales in high technology to the USSR and the socialist countries has existed for years, this is the first time the government has attempted to document its extent and significance. Citing such procedures as the creation of dummy firms, hand carried shipments without export licenses, and shipments made to illegal destinations, the document lists more than a dozen US companies who have engaged in this profitable but shady trade. Included are such small companies as I I Industries and

Kasper Instruments in California's Silicon Valley, which pleaded guilty in 1975 to diverting \$330,000 of semi-conductor equipment to the USSR by way of dummy firms in Canada, and Information Magnetics Corporation of Goleta, California, which shipped \$880,000 worth of computer supplies to Bulgaria through a UK subsidiary. Larger firms named in the document include Texas Instruments of Stafford, Texas, Tektronix Incorporated of Beaverton, Oregon, and Hewlett Packard of Palo Alto, California.

California gives major boost to solar energy

A major boost to the US solar energy industry has been given by a ruling from California's Public Utilities Commission that the state's four largest publicly-owned utilities must finance the installation of solar water heaters in 175,000 homes over the next three years.

The utilities — Pacific Gas and Electric Co., Southern California Edison Co., Southern California Gas Co., and San Diego Gas and Electric Co. — have been told to produce plans within 60 days for various types of low interest loans to solar-home owners.

Industry officials estimate that the commission's ruling will increase by more than five times that number of homes in the US with solar-assisted water heating. The plans still require further approval by the commission, and will be the subject of public hearings, but the PUC has indicated that it intends to achieve a major breakthrough in the acceptance of solar power by consumers.

"Certainly there isn't any question the PUC order is the biggest thing to happen to solar power", said Mr Edward A Myers, vice-president of Southern California Edison. Another utility official claimed that, as far as solar energy was concerned in California, "whether it is economically competitive really doesn't matter any more".

Average cost of installing the solar heaters is expected to be about \$2,500 for each house for equipment which would provide 50 to 70% of the energy needed for hot water.

First geothermal plant in Holland

THE FIRST geothermal project, part of a larger 32 million guilder energy research and development programme has been started in Holland. Fifty houses in Spijkenise, a Rotterdam suburb, will be connected in 1982 to a heating system which will circulate water at 100°C from 3000 metres underground. A unique aspect of the system is an interim storage procedure that will hold the water at a

depth of 600 metres during the summer months when demand is less. An estimated equivalent of 12-25% of Dutch natural gas reserves is available in geothermal energy. The most favorable source is in the south of the country where studies carried out since 1974 have shown gradients of 40-50° per 1000 metres of depth. **Caspar Schuurin**

US pays radiation exposed veterans

NINETEEN war veterans who were deliberately exposed to radiation from bomb tests in Nevada in the 1950s as part of a programme to show that the US fully intended to use its nuclear capability are receiving disability payments for cancer. But the great majority of 493 veterans who have filed claims for cancer contracted as a result of the tests have been turned down. Ten cases were awarded benefits and an additional nine were won on appeal but the Army denied the remaining 386 applications. Other cases are still pending.

UK nuclear inspectorate understaffed

THE UK's Nuclear Installations Inspectorate is facing increasing difficulty in meeting its obligations because of government spending cuts. Present staffing is 15% below full complement (17 vacancies out of 104 positions), and recruitment has proved difficult because industrial salaries are 30% higher and because of the Inspectorate's threatened move from London to Lancashire. In addition there are fears that the Health and Safety Executive may be forced to impose non-nuclear duties on its nuclear staff because of the impact of recent budget cuts. Nuclear inspectors are highly specialised and must be qualified engineers with 10 years' industrial experience. Mr Roger Gausden, Chief Inspector, has admitted in the NII's biennial report that "it has not proved possible to recruit the required additional staff to deal with all the tasks facing the Inspectorate".

University of Edinburgh rescinds "six year rule"

THE University of Edinburgh has rescinded its rule preventing the rehiring of untenured members of research staff after six years. Citing opposition to the "rigidity" of the rule, a report by a university working party last week said that "research staff are in the best position to make the decisions individually". The report cautions, however, that heads of departments should not make any "unjustified commitments". The "six year rule" has been rescinded "despite the fact that there now appears to be a tendency to adoption of a similar rule by some research

councils". The working party, chaired by Professor A G Mackie, surveyed the university non-tenured research staff and found that only half obtained permanent jobs on completion of their contracts, while 11% were unemployed and 32% had moved to other temporary jobs.

UNEP shames Mediterranean countries to pay bills

A MILITANT speech by United Nations Environmental Programme deputy director, Peter Thacher last week (*Nature*, 14 February page 613) has produced promises of payment by the delinquent participants in the Mediterranean clean-up programme. Italy has promised its \$745,000 arrears by April, Spain its \$412,000 payment by December and France its remaining \$573,000 "sometime this year". In addition cheques which have not arrived from Turkey, Yugoslavia and Monaco will be traced. The pollution programme will be forced to close in seven weeks if part of the money is not made good. In another major result at the three day meeting in Barcelona, Algeria signed the 1976 Barcelona pollution accords leaving Albania and Turkey as the only non-signatories. Algeria's signing signals important Third World support for the clean-up programme, expected ultimately to cost \$15 billion over 10-20 years.

France and India sign new technical agreement

FRANCE and India have signed seven new protocols for technical and economic cooperation which include research in renewable energies, oceanographic technology, and the development of semi-arid regions of the left bank of the Rajasthan Canal and the Bundelkhandmarea of Uttar Pradesh. Signed by French President Valéry Giscard d'Estaing on his visit to India last month, the protocols will also attempt to solve the long standing problem of Franco-Indian cooperation — the inability of India to make use of French credits because of the high cost of French equipment and technical service. **B. Radhakrishna Rao**

Sri Lanka to set up basic research institute

SRI LANKAN President J R Jayawardene announced last week to establish an institute for basic research to be housed at a site near Colombo by November of this year. The culmination of nearly a year of discussions between the President, local scientists, and several expatriate Sri Lankan scientists, the institute will emphasise advanced study in all branches of fundamental science. Professor N C Wickramasinghe, head of the department of mathematics at University College, Cardiff, UK, has been mentioned as a candidate for director of the institute.

FEATURES

Papua New Guinea (PNG) is one of the world's least "developed" countries. A third of its three million people have only emerged from the neolithic age over the past 40 years. The people are divided by mountain ranges reaching 4,700m, torrential rivers, forests, ravines, seas, malarial swamps and language — more than 700 are spoken. But in the five years since independence from Australia, the government has launched an ambitious Improvement Plan, under which western

science and technology are being introduced enthusiastically. All projects are funded by the National Public Expenditure Plan, which absorbs 21% of all spending. The main national aims are equal distribution of development among a population that is 85% rural and largely dependent on subsistence farming and a reduction in the number of western expatriates on whom development still largely depends. Both aims are meeting with mixed results, as **Tony Ades** reports

Appropriate technology:

PAPUA New Guinea's Eight Point Improvement Plan calls for self-reliance, less dependence for its needs on imported goods and services. Most conspicuous among imported services are those of the 4,200 expatriates employed by the government. While nearly all political posts are filled by nationals, expatriates have a dominant role in formulating policy and planning its execution.

Since self-government in 1973 the number of expatriates has fallen from 50,000 to 30,000, partly as a result of the localisation programme, the replacement of expatriates by nationals. In the public service, the proportion of expatriates has dropped by 10%, but there seems to be no hurry to enforce the planned 4% reduction per year because the number of qualified nationals is not increasing as fast as the nation's need for skilled administrators.

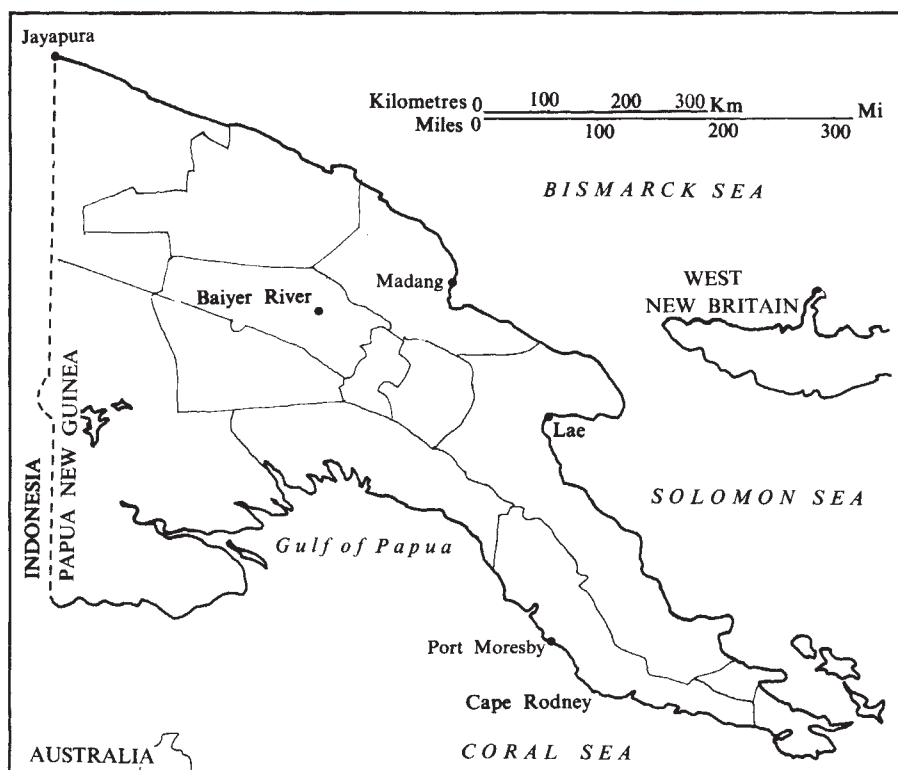
There are sound economic reasons for localisation. The average expatriate costs the government four or five times as much as the average national, and even at the same level the expatriate will earn between two and three times more. Also, their incomparably higher standard of living creates unfulfillable hopes in the local population. However, unlike most developing countries, PNG had no western-trained elite to take over at independence. Furthermore, the sheer inaccessibility of much of the country has brought education very unevenly. In the highlands, where many gratefully recall how the first Europeans ended interclan fighting after the Second War, people are

inclined to believe that localisation has been going too fast, leaving them permanently disadvantaged to the coastal peoples whose longer contact with the West and higher levels of education give them disproportionate political power.

Of all government services it is in fact education where localisation has been most dramatic. By 1972 all primary school teachers were nationals. Since then the number of teachers has doubled to 10,000. And although 60% of all children now attend primary school, there has been a serious fall in standards that will take many years to work itself out of the system. In the meantime, localisation of secondary school teaching is going ahead.

The standards problem is most severe in science and mathematics. Complaints that young men and women training to be Health Extension Officers or agriculture technicians cannot calculate drug dosages or areas are commonly expressed, and at all higher education institutions students take remedial maths courses that may delay their regular curriculum by up to a year. Whatever the economic and political advantages of localisation, PNG's ambitious plans for development demand so much gathering of data on every aspect of the land and population, and so much administration, that many are asking whether localisation too early has actually increased dependence on expatriates.

Tony Ades has a Nature writing fellowship and is currently at the PNG Institute for Medical Research, on leave from Sussex University, UK.



Papua New Guinea's view

An expansion of education and a consequent fall in average standards is not unusual in developing nations. In PNG it has led to demands, particularly from expatriate education planners, that a more merit-oriented system be introduced. At present, selection from one level of school to the next is based on a quota system biased toward children from lower quality schools and poorer regions. This policy is typical of the government's determination to redress regional imbalances. The government is equally unlikely to yield to suggestions that it drop its plan for 100% primary school enrolment by 1990, on the grounds that vitally needed extension services in health and agriculture are seriously hampered by illiteracy.

The concern for equality, rather than higher levels of education for a few, was based on the idea that PNG would adopt small-scale, labour intensive modes of development. At the time of independence there was a corresponding enthusiasm for "appropriate technology". But the Village Task Force, set up in 1974 to carry this through to the rural people, seldom progressed beyond the theoretical stage. Six years later, the South Pacific Appropriate Technology Foundation (SPATF) acts as an information clearing house and sets up small projects like village water supplies, but is still very limited in its capacity to create rural employment. Instead, SPATF has been set to work on the urban unemployment problem, and devises pilot projects such as furniture making.

Its research arm, The Appropriate Technology Development Unit at the University of Technology, Lae, is also more oriented towards hardware. It has designed a hydraulic ram pump that can be built with a drill press rather than an entire machine shop and is currently testing mini-hydroelectric schemes. The newly appointed Vice-Chancellor, Professor Alan Mead, has promised to involve more of the somewhat reluctant faculty in this type of work, but there exists as yet no channel by which rural needs and technical possibilities can meet each other.

SPATF's explicit policy of "not telling people what they want" demands that people have some idea of what is available. It has always been hoped that the increasing number of school leavers, teachers, extension workers, and even some university graduates would return to their villages and catalyse development. But those few who try are often rejected by the village elders. Extension workers are now generally posted away from their own villages, losing the advantages of shared language and culture. On top of this, a western education, even if below western standards, does not prepare one for a return to a subsistence lifestyle. A student at the University of Technology told me that he might eventually go back to his village, but only when electricity had reached it and when television broadcasting had begun. Then he would help the village develop by selling and servicing television sets. Such views are not unusual.

The fact is that the "appropriate

technology" emphasis on locally available materials and skills, and on the natural evolution of simple technologies at the hands of the craftsmen who use them, is scarcely relevant in a country with no traditions in metal, cloth or draught animals. For many the first wheel came on an aeroplane. The arrival of science and technology has been headlong: generally it is capital intensive and oriented to a high return. Papua New Guineans have welcomed sophisticated telecommunications, the F-28 jets flown by Air Niugini, and the highly sought after four-wheel drive land vehicles. And at the government level there is certainly room to doubt the commitment to appropriate technology. In spite of the recommendations in its own national paper to last years United Nations Conference on Science and Technology for Development, PNG operates no assessment or vetting of imported technical hardware.

On the other hand, in the field of energy, PNG enters the technological age with more foresight than any developed nation, and with a comprehensive policy aimed at self-sufficiency through renewable resources. The present expansion of the road system and the growing monetarisation is forcing up fuel imports at 8% a year. To counter this the initial step is to be a cassava-to-alcohol conversion plant in the Baiyer River area of Western Highlands Province. The 2 million litres produced each year will be mixed at 15-20% with motor spirit. The plant will be in a remote area not only to bring rural employment, but also to offset government subsidies for fuel transport costs, which run to K3.50 (\$4.62) per gallon in the most inaccessible regions. (One PNG kina is worth \$1.32.) If this conversion plant is a success, more will be built. It is hoped that the use of alcohol fuels will eventually reach a level that would justify the import of cars that run on 100% alcohol. At that point large scale wood-to-alcohol installations would be built, drawing on waste from forestry operations.

PNG has so far decided against rural electrification. Other aspects of the energy policy include pilot projects for charcoal production, wood pyrolysis, firewood cropping, together with research into solar air conditioning, sago to alcohol conversion, and biogas plants. Meanwhile, several small diesel generators are to be replaced with photovoltaics. The entire programme, the brainchild of Dr Kenneth Newcombe at the Department of Minerals and Energy combines all levels of technology with a sensitivity to rural skills and needs. It is also an outstanding example of how much scope expatriate scientists and economists have in moulding the course of PNG's development towards, paradoxically, self-reliance.

While many components of the energy policy accord with official approval of small-scale industry, and are therefore consistent with the educational system's stress on quantity rather than quality, the

course of development appears to be moving in the other direction, calling for more skilled management than is available. A shift away from smallholder projects is becoming evident. For example, cattle smallholdings are to be replaced with ranches owned by entire clans, worked by hired labour and large enough to pay the services of an expatriate manager. A flagging smallholder rubber development at Cape Rodney, Central Province, is to be revitalised by the addition of a central estate. The World Bank's Southern Highlands Development scheme also opts for nuclear estates. The Baiyer River alcohol plant will be supplied with intensively farmed cassava. The rice and sugar projects now under consideration not only have no place for smallholders, but are on a very large scale indeed.

The causes of this trend go deeper than economies of scale and the unstable output of smallholders. Officials concede that there has been insufficient effort to make the smallholder self-reliant. Extension officers have arranged for bank loans to farmers and taken most of the decisions to ensure that the loans are repaid. Effectively the smallholder is turned into a labourer on a government-managed farm. On top of this is the difficulty of extension work among a largely illiterate population with a chronic shortage of staff. Yet the government is committing only K300,000 (\$396,000) a year to adult literacy programmes. Moreover, Rural Development Assistants, who comprise the vast majority of agricultural extension staff, are to be phased out altogether. They will eventually be replaced with smaller

numbers of better-trained staff, but an increasing proportion of students graduating from agricultural colleges go into private business for themselves.

Eventually, the educational system will probably iron out the currently erratic course of development at the rural level, if, that is, its western orientation does not promote dissatisfaction with rural life. However, it may be perpetuating the lack of local expertise which recently led the Prime Minister Mr Michael Somare to try to drum up Australian investment. PNG is not at the mercy of foreign capital. Its laws clearly demarcate the areas in which investment is welcome, and it regulates the form that foreign business activities can take. But critics point out that this can only serve to amplify the gap between the still expanding subsistence population and the wage sector, and further disrupt the former.

In addition to this, the growing indigenous commercial interests, with their increasing political influence at provincial level, are also aiding the push towards a more capital intensive wage sector and away from subsistence and smallholder farmer. A related danger is that provincial governments are threatening to make their own arrangements with foreign companies, but have less ability to ensure favourable terms than the national government.

In accordance with its stated policies, Papua New Guinea is slowly achieving self-reliance as a nation, and redressing the inequalities between its regions. The question is: can it at the same time bring self-reliance and equality to its people? □

Eric Ashby (right) looks at a recent report on public participation in technology decision-making in OECD countries and argues that better public information could reduce disenchantment with representative democracy

THE WINDSCALE enquiry in Britain and the Mackenzie Valley Pipeline enquiry in Canada were highly publicised experiments in participation. They were responses to a demand which has now become insistent, for 'a greater degree of public accountability, freer public access to technical information, more timely consultation on policy options, and a more holistic approach to the assessments of impacts', to quote a report on participation from the Science Policy Division of the Organisation for Economic Co-operation and Development.

The report, *Technology on Trial*, by K. Guild Nichols, published in Paris last year, is a clear and useful account of the present state of the art of participation in OECD countries. The important point Nichols makes is that this demand is a symptom of a much deeper social disturbance — a disenchantment with the whole process of representative democracy.

The public elect people to represent their interests and straightway distrust them. Through the mass media self-appointed leaders who claim to represent the public interest can bypass the traditional hierarchy of procedure and appeal direct to the people over the heads of legislators. Nichols quotes Paul Valéry: 'all politics is based on the indifference of most of those concerned, without which politics would be impossible'. If Valéry was right, we are in for trouble; for concern, as measured by the number of people prepared to vote (for instance) in the referendum on atomic power held in Austria in November 1978, is spreading, and indifference to such great issues can no longer be assumed by those who make decisions.

Governments in pluralistic democracies are embarrassed by this surge of interest; as indeed they need to be, for this desire that the general public should participate in the decisions that affect their lives is undeniable in theory and confoundingly perplexing in practice. There is no such thing as monolithic public opinion. Even groups of people who agree about what is in 'the public interest' may do so for different reasons: one because he genuinely fears a nuclear economy; the other because he doesn't want a power station to spoil his view. The utilitarian calculus — that a social welfare function is the sum of a multitude of individual welfare functions — is a discarded and useless concept. The assumption, accepted for a long time by the man-in-the-street, that major

Depending on foreigners for self-reliance: expatriate manager (right) with cattle smallholders rigged out in clan gear



Participating in planning: the public needs better information



technological problems can be left to experts to solve, is no longer valid. If we want to remain a pluralistic democracy, we have got to invent reliable machinery for public participation.

Nichols' essay describes how information on technological issues is conveyed to, and elicited from, the public; and he describes how policy makers are informed and advised. He draws examples from several countries: Britain, the US, Canada, Scandinavia, Germany, Austria, France. He picks out the difficulties and displays them clearly. How can an unofficial group of citizens get access to the necessary information? Where can they raise the money to dispute a governmental policy? How can state officials, promoting what they earnestly believe is the public interest, be protected from vexatious, and sometimes mischievous, obstruction from groups acting out of self-interest? What are the comparative merits of agencies, legislative hearings, commissions, and the courts, as ways to resolve conflicts of values?

Nichols gives an accurate and objective summary of recent attempts in OECD countries to reconcile the conflicts of fact and value which are inevitable when nations have to make decisions about exploiting the environment. In just over 100 pages he could not be expected to cover the whole range of social experimentation that has been tried. This range has been classified, somewhat tendentiously, by S.R. Arnstein (in *The Journal of the American Institute of Planners*, xxxv, 1969, p.216) in six categories, grouped in pairs: (i) manipulation and (ii) therapy (designed to 'cure' the participants of what the proponents of a scheme deem to be ignorant prejudice); (iii) providing information and (iv) consultation (deemed by some participants to be 'tokenism'); (v) delegated power to participants and (vi)

citizen control (the two categories which Arnstein is prepared to regard as 'partnership'). Categories (i) and (ii) have been rejected by the lobbies which give themselves the duty of scrutinising the impact of technologies on the environment. As machines for public participation they are obsolete. Category (vi) would be regarded, by all but the most fanatical opponent of representative government, as unrealistic. Categories (iii) and (iv) are acceptable to some lobbies, and they could be made more widely acceptable if they were taken more seriously by governments.

For models of intelligent consultation combined with masterly presentation of facts we have to turn to Canada. Two outstanding examples are the reports of Mr Justice Berger on the gas pipeline from Alaska (summarised by Nichols); and the outstanding report from the Ontario Royal Commission on Electric Power Planning (*A Race Against Time: interim report on nuclear power in Ontario*, Toronto, 1978). The explanation of the CANDU fuel cycle in this report is a brilliant example of interpretation to the layman of the issues he must understand before he can play a useful part in any process of participation.

The corporation that generates electricity — the Ontario Hydro — has no statutory requirement to involve the public in its planning processes. Nevertheless, since 1972, the corporation has involved about a hundred people at head office and in its regions, holding public hearings, setting up citizens' committees, and listening to anyone who cared to talk to them.

Whether this has educated the public to understand the issues better is something that cannot be proved; but there is no doubt that the public have educated Ontario Hydro, and the Commission.

This, however, falls short of partnership in decision-making, and there are very few examples of the delegation of some power to the participants, Arnstein's category (v). Only one successful example of that is on record. It is to be found in a report prepared for the Electric Power Research Institute in California (*Proceedings of a Workshop on the Measure of Intangible Environmental Impacts*, EPRI Special Report, Palo Alto, 1976). The San Diego Gas and Electric Company had to site a new power station in southern California. They invited the public to set up a

committee of their own to examine alternative sites, provided them with financial help and expert advice, and left them alone to hold their own public hearings and to make a recommendation, which the Company accepted.

It is unlikely that participation in Britain will go beyond the levels of Arnstein's categories (iii) and (iv) in the foreseeable future; but if bodies like the Central Electricity Generating Board and the National Coal Board bring imagination and genuine enthusiasm to their schemes for providing information and for seeking consultation, the benefits even of limited participation could be considerable. First, it could restore some confidence in the judgement of those who inevitably have to make the final decisions (the process of decision-making is as important for society as is the wisdom of the decision itself). Second, it could encourage a sense of responsibility among the so-called public interest lobbies.

More participation will not necessarily clarify the issues themselves: indeed one of the surprising results of a massive experiment in public education about nuclear power in Sweden, financed from public funds and involving some 80,000 members of the public, was that at the end the number of people who felt unable to decide either for or against nuclear power increased from 63% to 73%. This is not — as some government officials asserted — a sign that the experiment failed; it is, rather, a sign that more people than before are aware of the immense complexity of the problems facing their representatives in government. This could increase public sympathy with decision-makers; and this in turn could help to turn the tide of disenchantment with representative democracy.

Our educational system must bear some of the blame for this disenchantment. In the last thirty years the impact of science on society has gone up by many orders of magnitude; but science at the popular level (for those who are not going to become professionals) is taught in very much the same old way.

We have not paid enough attention to telling people what they need to know about science if they are to perform their civic duties. (Often we tell people too much: Nichols quotes Thurber's little girl: "This book tells me more about elephants than I ever wanted to know".) The American science writer Philip Ritterbush put the challenge this way: '... scientific understanding of the public, rather than public understanding of science, might become the frame for discourse'. □

Lord Ashby is Chancellor of Queen's University, Belfast, Northern Ireland, and a fellow of Clare College, Cambridge, UK.

CORRESPONDENCE

Biotechnology and scientific collaboration

SIR, — In a recent issue of *Nature* (10 January, page 125) I was pleased to note in Robert Walgate's article the inclusion in the proposed EEC programme on biotechnology of "development of reactors for reactions at a hydro-organic interface", since our Biotechnology Unit has been responsible for several PhD theses and published papers related to this topic. The unit, set up several years ago, did effectively organise collaboration between biochemists, chemical engineers and microbiologists in this and other fields.

It was therefore surprising to read on the following page that "Lilly's group is unique in the UK in combining chemical engineers and biologists". While this group has indeed been pre-eminent in developing recognition of enzyme technology in this country, Dr Walgate could have ascertained that it was not unique, by reading the 1976 Report to the SRC and Institution of Chemical Engineers by A.N. Emery which cites at least four UK universities where such collaboration occurs.

A point of more concern is evidence of conceptual isolation of food science from biotechnology. Both involve application of the same basic disciplines of biochemistry, microbiology and chemical engineering and are similarly concerned with the provision, processing and utilisation of natural products. Both involve enzyme technology. This isolation is surely based more on territorial tribalism among applied scientists and their professional organisations than on a rational approach to the subject matter.

Now that the universities have entered lean years, where the relationships between the financial bones and the semantic skins are more carefully scrutinised, it is to be hoped that the various bodies concerned with biotechnological planning will not take too narrow a view of existing and potential assets.

Yours faithfully,

J.A. BLAIN

University of Strathclyde, Glasgow, UK

FAST's biotechnology programme

SIR, — Your issue of 10 January focusing on biotechnology was timely and relevant, in emphasising that "the middle-term problem rests with governments", and that "this is precisely the moment when patterns of research will be formed which will ultimately shape lives". The Commission's "FAST" programme (Forecasting and Assessment in the field of Science and Technology) was set up by decision of the Council of Ministers to highlight prospects, problems and potential conflicts likely to affect long-term development of the Community and define alternative courses of Community research and development action.

Biotechnology — or more broadly, a "bio-society" scenario — has been selected as one of our three central themes, and within this we shall be sponsoring a number of research studies covering such related long-term issues as Community research and development strategy, manpower and training implications,

social acceptability, Third World implications, and environmental engineering; further details are available on request.

I should point out that the FAST programme is distinct from the biomolecular engineering programme described in your issue of 10 January, though naturally we have the benefit of collaboration with our Commission colleagues, both from this programme and elsewhere in the Commission.

Yours faithfully,

MARK F. CANTLEY

Commission of the European Communities, Brussels

Shortage of science teachers

SIR, — It is reported in *Nature* (17 January, page 233) that there are serious shortages of teachers of mathematics and natural sciences, particularly physics, and it is revealed that of our secondary school teachers of physics only 43% are qualified to teach physics. Your report contains the wise and timely warning, "without well-qualified science teachers, there is little hope for the future of science and engineering in Britain". I wish to present the case that the situation is in fact even more serious than the figures suggest.

Firstly, I question the suitability of the education of many (or probably, most) of those who are "qualified to teach physics". University graduates generally have acquired an imperfect knowledge of advanced work in a specialist subject. To teach physics in school one requires a very thorough understanding of the elementary principles of the science, and also considerable knowledge of the relationship between physics and other subjects.

Secondly, there is a tacit assumption in all educational thinking that teachers and pupils can easily obtain trustworthy information about any subject by reading textbooks. It is now 20 years since the writer first drew attention to the incredible accuracy of very many physics textbooks, particularly at sixth form level. During the past 20 years a very few publishers have attempted to improve their own books, but the general standard has continued to decline.

As it is obvious that we cannot quickly remedy the shortage of suitably trained teachers, surely it is obvious that we must help the teachers we have by ensuring that they have accurate sources of information.

Yours faithfully,

J.W. WARREN

Brunel University, Uxbridge, Middlesex, UK

Brazil's fuel technology

SIR, — I was very interested in the article on alternative fuel technology in Brazil (6 December, page 550) which you published recently. Unfortunately, in the discussion of ethanol production, the author did not answer the one question which seems to me the most important in this whole project. Where does the fuel come from to distil the ethanol? By fermentation, ethanol can only be produced in dilute aqueous solution, and the amount of energy needed to fractionally distil this may be greater than the energy obtained from burning the ethanol.

It seems to me that this need for an energy input to fractionate the ethanol may be a fatal weakness in the whole ethanol project. It is avoided by the use of methanol, which is not produced in dilute solution, and may be made, for example, from wood via charcoal.

Yours faithfully,

DAVID A.H. TAYLOR

University of Natal, Durban, Natal

Nuclear power

SIR, — There seems to have been a serious misprint in the penultimate paragraph of the article on *Nuclear Power: the threat to personal freedom* in the 20-27 December issue (page 774). The paragraph states that the pursuit of nuclear power could conceivably damage civil liberties less than would the pursuit of a high conservation/low energy strategy as outlined earlier this year by the International Institute for Environment and Development. Since the IIED strategy does not touch personal liberties and at most calls for mandatory targets to be set for the energy performance of products such as cars and new buildings, thus merely altering the range of consumer choice in one respect, the paragraph is clearly back to front. Energy conservation may have to be forced through by 'controls' on individuals only if we do 'too little, too late'; but no critic has yet accused the IIED scenario of that! And even controls of this kind are a long way from armed police and security vetting.

Yours faithfully,

GERALD LEACH

International Institute for Environment & Development, London, UK

Job-swapping

SIR, — In regard to your editorial on job-swapping (4 October, page 327), I had the good fortune to exchange jobs and houses with a librarian in Scotland for an academic year and would do it again eagerly.

It did, however, require a fairly substantial amount for travel expenses and salaries, which therefore were paid by our home institutions. I think your point about the necessity for a 3-5 year tenure is unnecessary and, on the contrary, for family reasons, I would suggest a 2-3 year exchange.

Yours faithfully,

DANA L. ROTH

California Institute of Technology, Pasadena, US

New Jersey Turnpike

SIR, — Robert Claiborne (20/27 December, page 776) says that my "attitude to facts is not beyond criticism", but his basis for this statement is manufactured out of context. I said that on a specific occasion the odors and visual obscuration ("like twilight or an eclipse of the sun") described in the book did not occur. The book implied (p102) that this alleged nuisance, caused by the large petrochemical complex near the New Jersey Turnpike, is not even occasionally abated. I lived near the complex for 21 years, and the statement in the book is incorrect.

Yours faithfully,

THOMAS H. JUKES

University of California, Berkeley, US

NEWS AND VIEWS

Physics at very high pressures with laser-driven shock waves

from M.H. Key

RECENT papers by Trainor *et al.* (*Phys. Rev. Lett.* **42**, 1154; 1979) and by Veerer *et al.* (*Appl. Phys. Lett.* **35**, 761; 1979) highlight the fact that in relatively simple experiments laser-driven shock waves can be used to study the propagation of shocks in solids for shock pressures up to terapascals (1 TPa = 10^7 bar = 10^{13} dyne cm^{-2}). In view of the fact that the only demonstrated alternative for studying such high pressure shock waves requires a nuclear explosion (Ragan *et al.* *JAP* **48**, 2860; 1977) the simplification provided by the laser approach is considerable.

Laser irradiation of a solid surface in vacuum creates a high pressure surface plasma. The pressure exerted on the solid by this 'ablation plasma' is shown in Fig. 1 as a function of laser irradiance I and for two laser wavelengths $\lambda = 0.53$ and $1.05 \mu\text{m}$. The ablation pressure acts as a 'piston' driving a shock wave into the undisturbed solid. Accurate shock velocity measurements were obtained by Trainor

et al. and Veerer *et al.* by recording the differential time of transit of the shock through $\sim 30 \mu\text{m}$ thick foil targets with a step variation in thickness as in Fig. 2. The arrival of the shock at the rear surface was signalled by a fast ($\sim 10^{-11}$ s) rise in luminosity detected by an electron optical streak camera with time resolution $\sim 10^{-11}$ s.

The maximum shock pressure for which these techniques are useful is limited by the saturation in the rate of increase of ablation pressure with intensity (Fig. 1) and the associated increasing depth and magnitude of target preheating due to energetic electrons generated in the process of absorption of the laser light in the surface plasma. Where pressure due to this preheating becomes significant relative to the ablation pressure and the preheat penetration depth becomes comparable with the target thickness the process no longer results in a simple shock propagating in an undisturbed medium. Investigation has shown that the preheating depth is a function of $I\lambda^2$ and that the maximum value of $I\lambda^2$ for simple shock generation in these targets is of the order of $3 \times 10^{14} \text{ W cm}^{-2} \mu\text{m}^2$ (Hares *et al.* *Phys. Rev. Lett.* **42**, 1216; 1979). Thus shock pressures up to 1.8 TPa were reported by Trainor *et al.* using $1.06 \mu\text{m}$ radiation while Fig. 1 suggests that with $\lambda = 0.53 \mu\text{m}$ pressures up to about 10 TPa should be attainable.

The motivation for these studies is that the equation of state of compressed solids is not well known in the interval between a few tenths and a few tens of terapascals. At lower pressures extensive experimental data from static high pressure and chemical explosive experiments are available while at higher pressures the relatively simple Thomas Fermi (TF) theoretical model is valid (Ragan *et al. op. cit.*). The TF model neglects all atomic structure and quantum

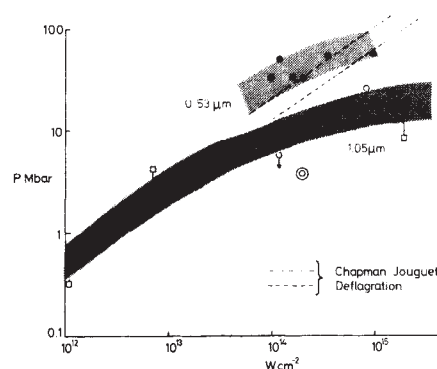


Fig. 1 Ablation pressure in units of megabar as a function of laser irradiance for 0.53 and $1.05 \mu\text{m}$ laser wavelength. (Dashed lines theoretical model). Unpublished results obtained by research groups from Imperial College London and Queen's University, Belfast working at the SRC Central Laser Facility, Rutherford Laboratory.

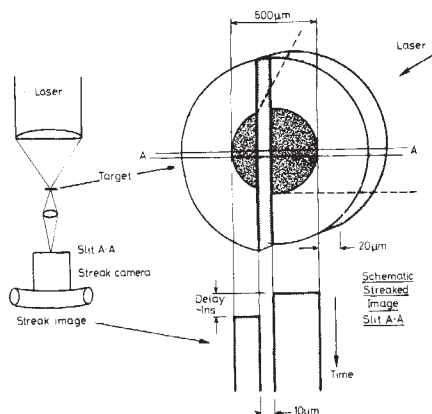


Fig. 2 Schematic diagram showing how streak photography is used to measure shock velocity in a planar foil target with a thickness discontinuity.

M.H. Key is at the Rutherford Laboratory.

mechanical binding force between atoms and treats only the compressibility of a degenerate electron gas in the field of positive nuclei. At the opposite extreme a solid at zero pressure is in equilibrium between the degenerate electron repulsive forces and interatomic binding forces. Theoretical analysis for conditions where the applied pressure is not large relative to the binding forces is complex and experimental studies are needed.

The laser technique of shock generation extends the upper pressure limit for such measurements since measurement of any two of the five shock parameters (material density, pressure, internal energy, flow velocity and shock front velocity) allows solution for the other three (from the conservation of energy momentum and mass flow across the shock front) and hence gives the equation of state relationships between pressure, density and internal energy (Ragan *et al. op. cit.*).

Intermediate filament cycles

from Brian Anderton

RESEARCH into the structure and organisation of the distinct fibrous organelles which make up the cytoskeleton of eukaryotic cells has intensified enormously in the past four or five years. The latest component of the cytoskeleton to come under close scrutiny is the intermediate filament. Intermediate filaments are approximately 10 nm in diameter and are so called because their width lies between those of the other two cytoskeletal fibres, microtubules (24 nm) and microfilaments (6 nm). Unlike tubulin and actin, which are the principal structural proteins of microtubules and microfilaments respectively, intermediate filament proteins seem not to be highly conserved and show a certain degree of tissue specificity. They have been divided into subclasses on the basis of biochemical and immunochemical data and with the inevitable consequence of isolation, the constituent proteins have been given names (in some cases more than one).

Five subclasses have been identified and new ones may need to be added. So far the (roughly tissue-specific) subclasses comprise (1) prekeratin tonofilaments of epithelial cells; (2) desmin or skeletin filaments of smooth muscle which are also identified with the protein present in the z-line region of cardiac and skeletal muscle; (3) filaments of fibroblasts and other mesenchymal cells (vimentin or decamin); (4) neurofilaments of neurones; and (5) glial filaments (glial fibrillary acidic protein) present in astrocytes (but not other glial cells). Structural homologies between different intermediate filaments have only occasionally been sought but some do seem to exist (Gard *et al.* *Proc. natn. Acad. Sci. U.S.A.* **76**, 3894; 1979; Steinert *et al.* *Proc. natn. Acad. Sci. U.S.A.* **75**, 6098; 1978).

The biochemical characterisation of different intermediate filaments has been hampered by the fact that when isolated they are mostly insoluble in salt solutions. This unfortunate physical behaviour from the point of view of the biochemist now seems to have been overcome by Zackroff and Goldman (*Proc. natn. Acad. Sci. U.S.A.* **76**, 6226; 1979). Goldman and his colleagues have for some time been studying the fibroblast intermediate filaments from cells of the baby hamster kidney line BHK-21 and have shown that the main constituents are two polypeptides with chain weights of 54,000 and 55,000 which they have called decamin. In their latest paper they report a detailed investigation of an earlier observation that BHK-21 intermediate filaments disassemble on prolonged exposure to solutions of low ionic strength at around

neutral pH and reassemble on returning the protein to physiological ionic strength. When disassembled the material seems to consist of short rods or protofilaments perhaps 50 nm long and about half the width of an intermediate filament; the authors make no estimate as to the number of polypeptides constituting a single protofilament.

Turbidimetric measurements during reassembly show the process to be biphasic. Examination by electron microscopy suggests initial lateral association of the short thin rods to produce short thick rods followed by elongation of the latter to give apparently reconstituted intermediate filaments. The assembly process exhibits a critical protein concentration for initiation in the range 0.05–0.15 mg ml⁻¹. Possibly the most significant aspect of the work is the discovery that cycles of disassembly/reassembly can be achieved, although protein recovery seems problematical, and that after two such cycles the stoichiometry of the 54K and 55K polypeptides seems to remain around unity and several high molecular weight proteins above 250,000 copurify with the decamin polypeptides. This approach of inducing cycles of polymerisation/depolymerisation has been used successfully for purifying tubulin and identifying microtubule-associated proteins which remain in a constant weight ratio to the tubulin through repeated cycles. No doubt this report from Goldman's laboratory will now encourage others to apply the same technique in attempts to define the molecular composition of intermediate filaments from different sources. It should be pointed out, however, that Zackroff and Goldman believe the large decamin-associated polypeptides not to be essential for intermediate filament assembly since their removal by mild proteolysis does not block polymerisation of the 54K and 55K decamin polypeptides.

It remains to be seen whether or not the BHK-21 fibroblast intermediate filaments are heteropolymers of the 54K and 55K chains or if homopolymers of each exist separately inside the cell. Lazarides and colleagues have suggested that muscle intermediate filament protein, which they call desmin, is not restricted to muscle in its distribution but is also found in non-muscle cells such as fibroblasts, including BHK-21 cells (*Proc. natn. Acad. Sci. U.S.A.* **76**, 3894; 1979). They have assigned the 54K polypeptide from BHK-21 cells to desmin and the 55K polypeptide to the true fibroblast intermediate filament protein. They have also recently claimed that fibroblast intermediate filament protein too is more widely distributed than hitherto assumed and that it occurs along with desmin in the myofibril z-disc (*Cell* **18**, 1053; 1979). Notwithstanding earlier reports that desmin was absent from non-muscle cells, these more recent data seem convincing and are analogous to the results from Franke's, Weber's and Osborn's

laboratories which show that epithelial cells also contain two types of intermediate filament, namely prekeratin tonofilaments and fibroblast intermediate filaments for which they use the name vimentin filaments (*Differentiation* **14**, 35; 1979). However, more studies are needed as the BHK-21 cell line may not be typical. It is unclear, for instance, whether mouse 3T3 cells contain two types of intermediate filament polypeptide as published electrophoretic patterns (Franke *et al.* *op. cit.*) of purified intermediate filament protein from these latter cells show a single prominent polypeptide and not a doublet like that found from the BHK-21 cells.

Although such ambiguities remain, the classes and distribution of intermediate filaments are now beginning to fall into a pattern although we remain mostly in the dark as to their function. Probably the predominant view is that they are important in maintaining cell shape and the spatial organisation of other intracellular structures. However, now that they promise to be amenable to biochemical investigations of the type used by Zackroff and Goldman, their lack of established function is unlikely to deter the protein biochemists. □

Kimberlite and carbonatite magmas

from K. G. Cox

THE Earth's crust is a relatively thin layer made up of familiar rocks similar to those seen at the surface. However, at a depth of about 10 km in oceanic areas and at 50 km beneath the continents a sudden increase in the velocity of seismic waves marks a fundamental change of structure. From here downwards, to the outer edge of the core at a depth of about 3,000 km, the rocks are referred to as the mantle. This is volumetrically by far the largest compositional component of the Earth but relatively little of a direct nature is known about it. The mantle 50 km below our feet is distinctly less accessible than the Moon. Apart from the very uppermost levels of the mantle, which are occasionally exposed at the surface as a result of violent continental collisions, the only samples available are brought to the surface as fragments ('nodules') suspended in magma during volcanic activity. The distinctly rare volcanoes known as kimberlite pipes, which of course also supply diamonds, are the richest source of such materials.

At a recent meeting in London* J.B. Dawson (University of Sheffield) gave an authoritative review of the present state of knowledge of upper mantle rocks derived as nodules from kimberlite pipes, largely from southern Africa.

*The Anniversary Meeting of the Mineralogical Society, held in London on 10 January focused on the petrology of mantle materials and deep-seated magmas such as kimberlite and carbonatite.

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The most abundant nodule type is a very magnesium-rich silicate rock termed garnet peridotite and consisting largely of the minerals olivine and enstatite with smaller amounts of garnet and diopside. A distinction can be made between peridotites which are 'fertile' as opposed to 'barren', by which is meant their capacity to give rise to basaltic silicate liquids, the most common surface manifestations of volcanic activity. Barren peridotites are depleted in the low melting components calcium and aluminium, poor in garnet and diopside as a consequence, and seem therefore already to have undergone melting episodes to give rise to basalts. Most of the upper mantle sampled by kimberlites seems to be of this type and has evidently been involved in the melting processes of the past by which the crust has become separated from the mantle.

A distinction is also made between sheared and unsheared peridotite nodules. The former provide evidence of deformational events within the mantle, and it was postulated some years ago that they represented samples of the asthenosphere, that is the supposed layer of mobile mantle underlying the Earth's rigid surface plates. However plate motions as measured by rates of continental drift seem to be far too slow to account for the very strong deformational fabrics observed, and current opinion is drifting towards an alternative theory that the deformation is purely local and connected with the uprise of the kimberlite magma itself.

The study of nodules is the most direct way of obtaining information about the mantle. However many volcanic rocks are the solidified products of melts which originated in the mantle, and so provide another important approach to mantle problems. As well as kimberlite the extraordinary igneous rocks known as carbonatites are an important source of information. These are unique in that they solidify from carbonate, not silicate, melts. The session on kimberlite and carbonatite magmas tackled first the question of whether the two were related. Kimberlites occasionally contain a good deal of magmatic carbonate, both sorts of magmas are emplaced as relatively small intrusions in continental areas and occasionally show a fairly close relation in space and time and both are characterised by concentrations of relatively rare elements. R.H. Mitchell (Lakehead University, Ontario) maintained that because spinel mineral compositions in kimberlites and carbonatites are different the idea of a petrogenetic relation between the two rock types is not supported. S.E. Haggerty and B.M. McMahon (University of Massachusetts, Amherst) disagree, concentrating on mineralogical evidence suggesting that magmatic calcium carbonate was an integral part of kimberlite. Clearly at this stage a definition of what is meant by the term carbonatite is

required. Does for example the carbonate component within kimberlite count as carbonatite, or is it simply carbonate? However the lack of definition on this topic is probably less important than the failure to define what is meant by the term petrogenetic relationship. The relationship could consist of a common source material, a common magmatic precursor, a common thermal or deformational event, the exploitation of a common structural weakness in the crust, or some combination of these. W.J. Verwoerd (University of Stellenbosch, S. Africa) in describing the remarkable sequences of aligned intrusions in Angola, S.W. Africa/Nambia and the western part of Cape Province suggested perhaps wisely in view of the complexity of the question, that possibly the only common factor relating the kimberlites and carbonatites in this area was their exploitation of the same structural features. In areas where kimberlites and carbonatites are less common, for example in south east Australia as reviewed by J. Ferguson (Bureau of Mineral Resources, Canberra) and in N. America as reviewed by H.O.A. Meyer (Purdue University, Indiana) any relationship between the two rock types is difficult to find. However the rather close spatial and time relationship between the Murfreesboro kimberlite and the Magnet Cove carbonatite in Arkansas remains an intriguing coincidence.

Concerning the generation of kimberlite magmas D.K. Bailey (University of Reading) produced an ingenious and stimulating hypothesis. He pointed out that continental geotherms (distribution of temperature with depth) deduced from kimberlite nodules from different localities were all closely similar and that these could lie in 'grazing coincidence' with vapour-saturated solidi (onset of melting curves) of mantle rocks. Thus the introduction of fluids (such as water) under such conditions could initiate melting in a very restricted depth range and give rise to kimberlite magmas. Such magmas can only reach the surface if they erupt very rapidly since any attempt to migrate slowly upwards while losing heat results in their solidification. If the thermal gradient is lower than the critical value no melting occurs. On the other hand if it is higher, melting starts at a lower level and the magmas can migrate upwards without immediate solidification, interact with overlying mantle and lose their kimberlitic character. Bailey thus proposes that "kimberlite activity is the limiting case of cratonic magmatism".

On the subject of carbonatites I.C. Freestone and D.L. Hamilton (University of Manchester) reported successful experiments demonstrating immiscibility between natural soda-rich silicate liquids (phonolite and nephelinite) and natural alkali carbonatite. M.J. Le Bas (University of Oxford) is in the Department of Geology and Mineralogy, University of Oxford.

of Leicester) also stressed the importance of alkalis in carbonatites pointing out that deduced compositions of fenitising fluids (fluids which transform wall-rocks) and observed compositions of fluid inclusions in calcic carbonates agreed in indicating a significant alkali-metal content.

Much presented at the meeting gave food for thought but probably nothing more so than the account by A.R. Woolley (British Museum) of his re-mapping of the Alnö carbonatite complex. Brogger's work on Fen and von Eckermann's on Alnö are the historical foundations of carbonatite geology, and yet von Eckermann seems to have been wrong on many important points. However, wrong though von Eckermann may have been, he did recognise many important phenomena and that the carbonates were igneous not sedimentary, so Alnö played its part in the development of the science notwithstanding. □

Antigravity?

from P.C.W. Davies

NEUTRALISING the effects of gravity has long been a preoccupation among Tibetan monks, yogis, UFOlogists and science fiction writers. On the other hand, Einstein's theory of relativity — the best available theoretical description of gravity — leaves little room for antigravity. This beautiful theory, however, is believed by many physicists to be only an approximation, the reason being that it is inconsistent with the twentieth century's most powerful physical idea — quantum mechanics.

Attempts to unite mathematically Einstein's gravity with quantum mechanics have been frustrated for decades by daunting technical and conceptual problems. Recently, though, a promising new approach has emerged. Known as supergravity, the new theory attempts to treat the familiar gravitational field as only a component part of a more elaborate network of forces and fields. It is within this wider network that hitherto unsuspected forces could arise to neutralise gravity.

Quantum mechanical theory is a set of principles that governs the subatomic world and supergravitators think of gravity in terms of subatomic particles. The ordinary force that keeps our feet on the ground is interpreted in this theory as due to a continual exchange of a hypothetical quantum particle called the graviton. The graviton is gravity's counterpart to the photon of electromagnetism. A crucial difference between gravitons and photons is that the former has twice as much spin as the latter, which can be shown to account for the fact that like electric charges repel, whereas like massive bodies attract. In units of photon spin, the graviton has spin 2.

For comparison, the elusive neutrino has spin $\frac{1}{2}$, as do electrons and protons, whereas pi-mesons have no spin. The amount of spin carried by a subatomic particle is crucial to the type of mathematics used to describe it. Particles like mesons, photons and gravitons, with integral units of spin, enjoy certain geometrical symmetries that are different for the half-integral spin particles. For many years physicists regarded these two categories of particle as quite distinct forms of matter and energy. Then, in the 1960s, when the variety of subatomic particle species grew bewilderingly fast, it was discovered that many particles hitherto regarded as different could, in a certain mathematical sense, be envisaged as simply different faces of the same basic unit. Physicists began to group the particles into families: the neutron and the proton, for example, became part of an octet of particles that can be thought of as eight slightly different manifestations of just one particle.

In the early schemes, the integral and half-integral spin particles were always kept in separate families because of their very different mathematical symmetries. But in the 1970s several people proposed arrangements in which even those two groups could be mixed together in still larger families, enjoying a new, all-embracing, 'supergravity'. It is from this development that supergravity has arisen. Early models of supergravity put the spin 2 graviton in a small family unit with a completely new particle — the more hypothetical gravitino — having spin $3/2$. Soon this model was extended to a family with one spin 2 graviton, two spin $3/2$ particles and a single spin 1 particle, like the photon. It is this latter entity that could be responsible for antigravity.

Writing in a recent issue of *Physics Letters* (88B, 265; 1979) J. Scherk of the Laboratoire Normale Supérieure in Paris, examines the feasibility of the antigravity idea within the context of an extended supergravity model. Basing his analysis on earlier work by K. Zachos of Caltech (*Phys. Lett.* 76B, 329; 1978), Scherk points out that circumstances could arise in which the spin 1 component of supergravity exactly neutralises the spin 2 gravity. At first sight, the theory seems to predict that the gravitational force between neutrons should be exactly neutralised by spin 1 antigravity. This would render the theory nonsensical — more than half the mass of the Earth is in the form of neutrons. However, the way in which gravity and antigravity affect the matter might be different. Closer examination shows that while ordinary gravity responds to the entire mass of a body, for example a whole neutron, antigravity sees only the elementary constituents. Particles such as neutrons and protons are believed to be

composites of three so-called quarks.

Paradoxically, the assembly of three quarks does not have the same mass as three individual quarks. This is because enormous energies are released when the quarks bind together, and this energy also has mass. Taking this into account, Scherk estimates that the effects of antigravity would suppress the Earth's gravitational force on a body by about one part in a million, depending on the proportion of neutrons to protons in the substance concerned. This is well above the experimental upper limit obtained by comparing the rate of fall of differently constituted bodies.

To rescue antigravity from the problems of its own strength, Scherk speculates that although the antigraviton is described by the theory as massless, implying that it travels at the speed of light, it could acquire a small mass spontaneously as a result of a mechanism called symmetry breaking. The upshot of this idea is that the antigraviton is slowed up and the antiforce becomes short in range — perhaps extending for only a couple of metres. It is also greatly depleted in strength. Though this mechanism thereby renders antigravity utterly insignificant under most circumstances, Scherk remarks somewhat tongue-in-cheek that at a high enough temperature the symmetry breaking would fail, and antigravity could be important. The snag is, the temperature concerned would have to be well in excess of billions of degrees. So much for levitation! □

The closest cosmic radio source

from Alan Johnstone

VERY few of the radio sources discovered by radioastronomers will ever be visited by spacecraft carrying instruments capable of observing directly the electromagnetic fields and charged-particle distributions which generate the radio waves. Jupiter and Saturn are likely to be the only cosmic radio sources, observable from the ground, where *in situ* plasma measurements can be made. If some spacecraft were able to observe a celestial radio transmitter from within, the information would be extremely valuable, first because it would considerably improve the chances of understanding the source mechanism and second because the resulting knowledge could then be applied to more remote situations where there is no chance of *in situ* measurements.

Fortunately there is no need to send a spacecraft all the way to Jupiter to make these measurements; the closest such radio source is only a few thousand kilometres above the Earth's surface, where radio noise in the kilometric wavelength range

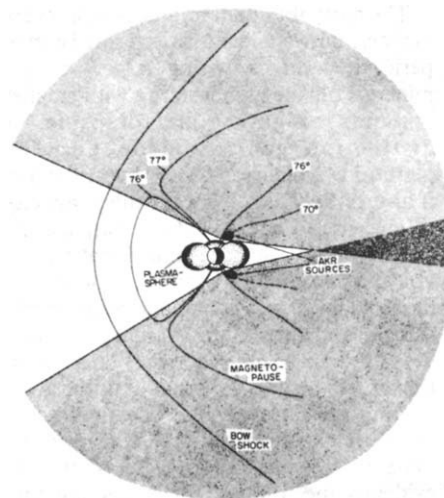


Fig. 1 The time-averaged extent of the conelike emission of auroral kilometric radiation superimposed on a magnetic field model. On the average both sources are observable in the equatorial plane on the nightside beyond $12 R_E$ (Gallagher & Gurnett *op. cit.*).

(100 kHz — 1 MHz) is generated. Unlike most of the radio noise created in the Earth's magnetosphere it escapes outward from the source region into space because the frequency is above the local plasma frequency. It cannot be detected on the Earth's surface because the frequency is below the peak plasma frequency in the ionosphere, so that all the radiation which starts downward is reflected back out again. The power radiated from the Earth is extremely variable, averaging 10^7 W and reaching peaks in excess of 10^9 W, making it comparable with Jupiter as a radio source. At its maximum the radiated power is 1% of the rate at which energy is being dissipated in the atmosphere by precipitating electrons during an auroral display. This comparison is important because the radiation seems to come from the region where auroral electrons are accelerated and to occur only at times when there are active, discrete, auroral forms visible in the auroral oval.

It is difficult to locate the source accurately from a spacecraft since an appropriate antenna consists typically of a single, long electric dipole which does not provide sufficient directional information. So far, two methods have been used. The first used the fact that the spin-modulation of the signal strength is a minimum when the antenna is pointed towards the source (Gurnett *J. geophys. Res.* 79, 4227; 1974). The minimum had an angular width of 6° when the source was observed looking back toward the Earth from a distance of $32 R_E$ Earth radii ($32 R_E$). This placed the source close to Earth, but did not locate it precisely. The second method used the occultation of the source by the Moon as seen from a spacecraft in orbit around the

Moon (Alexander & Kaiser *J. geophys. Res.* **81**, 5948; 1976). They found that the source is strongest and most common in the local time sector from 21 hours to 24 hours, the time at which auroral arcs are most common. From the Moon at first quarter the night-time magnetic field lines are seen in profile. The source of the auroral kilometric radiation then seems to lie along high-latitude field lines within a few R_E of the Earth's surface.

Gallagher and Gurnett (*J. geophys. Res.* **84**, 6501; 1979) have used a totally different approach. They collected data from two satellites for a total of 7 years and found the average intensity of the kilometric radiation as a function of radial distance, local time and magnetic latitude. Despite the variability of the source strength, they find that the average intensities are meaningful in tracing the source. The average intensity integrated over geocentric spherical shells (to find the total outward flux) is negligible below $2.5 R_E$, rises to maximum at $4 R_E$ and then remains essentially level to $30 R_E$. This places the source between $1.5 R_E$ and $3 R_E$ above the surface. The radiation originates at auroral latitudes, between 22 and 24 h local time, spread out over a wide cone (Fig. 1). The average intensity decreases outwards from the source as $1/R^2$ if it is assumed to be at an altitude between $1 R_E$ and $2 R_E$.

This is now known to be where auroral electrons are accelerated by several thousand volts, possibly by an electric field parallel to the magnetic lines of force. There can now be little doubt that the kilometric radiation is related either to the acceleration process itself or to the interaction between the beam-like electron velocity distributions and the cooler ionospheric plasma. Since the radiated power is as high as 1% of the energy of the accelerated electrons, the mechanism — whatever it is — must be a coherent and efficient process. There is no generally-accepted theoretical explanation and a great deal of effort is being expended to find one. There have only been a few *in situ* measurements in the region of interest, despite its relative accessibility, although the number should soon be increased by the NASA Dynamics Explorer Satellites. There should be advances on this scientific front before long.

It is clear that it would have been very difficult to have deduced the type of electron velocity distributions occurring in the source region from the characteristics of the radiated spectrum alone. This emphasises the importance of *in situ* measurements in unravelling the working of complex plasma processes. Many important and common plasma processes occur nearby in the magnetosphere where such measurements can be made easily. Auroral kilometric radiation is similar in spectral and temporal structure to the decametric radiation from Jupiter and the hectometric radiation from Saturn (Kaiser & Stone *Science* **189**, 285; 1975) so lessons

learned in the magnetosphere can be applied to Jupiter and Saturn and then perhaps extended to more remote sources.

A new look at the Sherborne bone

from Ann Sieveking

IN a recent number of *Antiquity*, (53, 211; 1979), R.A.H. Farrar has put forward a case for reconsidering the status of the Sherborne bone. This small engraving of a horse's head and forequarters was published by Arthur Smith Woodward in 1914, as apparently Palaeolithic in date but, 10 years later, was dismissed by Professor Sollas in the third edition of his book *Ancient Hunters and their Modern Representatives* as a forgery (see *Nature* **117**, 86, 233; 1926). The controversy continued in *Nature*, with Sollas emerging as the victor, although more by Smith Woodward's default than by the production of any conclusive proof. The question has been re-examined from time to time, at least with regard to the alleged evidence of hoaxers, or hoaxed, but little attention has been given to the bone itself, except for an analysis of its degree of fossilisation, made by K. Oakley in 1957. Some of the protagonists in fact, apparently including Sollas himself, never looked at the original.

There is little evidence to support a renewed claim for authenticity however. Such evidence as we have about the discovery of this engraved bone is hearsay, contradictory and unacceptable; its provenance, whether it was found in a heap of quarry stones, or in a rubbish heap beside the Bristol road, is without context and its intrinsic characteristics lend little credibility to a Palaeolithic attribution. At this stage the only useful contribution one may make to the problem is in examining the bone itself and here it must be emphasised that it is not the antiquity of the bone that is in question, (Oakley's analysis confirmed it as being fossilised) but the age of the engraving on it.

Smith Woodward maintained that the surface of fossilised bone would flake when engraved and that the Sherborne horse, cut with a relatively clean line, must therefore have been engraved when the bone was

fresh. However, tests made by M.H. Newcomer at the Institute of Archaeology do not support this claim: as clear a line may be cut with a flint burin on fossilised bone as on fresh. Further analysis of the technicalities of the engraving (for which observations I am grateful to Dr Newcomer) demonstrates that the surface of the Sherborne bone shows no preparation before engraving, although this is a common feature on Palaeolithic pieces. Under magnification it can be seen that, at certain points, where the engraved line crosses a natural crack in the bone, the cut dips down into such a crack whereas a line engraved before any cracking occurred would maintain an even depth. In addition, the engraved lines of the head are discontinuous and hesitant which, although in no way conclusive is, again, uncharacteristic of Palaeolithic work. Lastly, in the region of the mane of the Sherborne horse, the original surface of the bone has flaked away in antiquity, leaving a rougher surface below. The engraved lines of the mane, however, start on the smooth original surface of the bone and continue onto the rougher flaked surface (See Figs 1 and 2) at the edge. Had the engraving been contemporary with the fresh bone, these lines could be expected to have been truncated by the flaking, and the fact that they are not suggests that the engraving has been added subsequently to an already degraded bone.

Finally the stylistic characteristics of the Sherborne engraving must be considered. Farrar remarks that the Sherborne horse has an upstanding mane and lacks clearly defined ears, both of which characteristics may be paralleled in Palaeolithic examples, for instance in the Creswell Crags engraving (in the British Museum collections) and that less characteristically the Sherborne horse shows no linear definition below the mane and has an unusually drooping muzzle. He cites a parallel to this atypical muzzle in an illustration in Graziosi (*Palaeolithic Art*, 1960; Pl. 134.b); this illustration, however, is a drawing and not a photograph of the original engraving in the cave of Pindal and in this respect of the drooping muzzle it is inaccurate. The engravings of horse heads from La Grande Paroisse, Pincevent and from Cépy in Loiret, which are closer than is Pindal in date, location and the form of support used to the English Upper Palaeolithic art pieces, show an animal with a short, broad

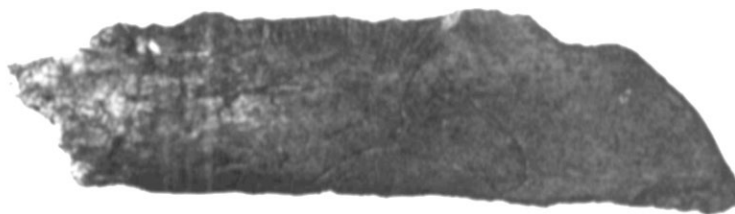


Fig. 1 The Sherborne bone (actual size ~8.5 cm), now in the collections of the British Museum (Natural History). Photo: Jill Cook.

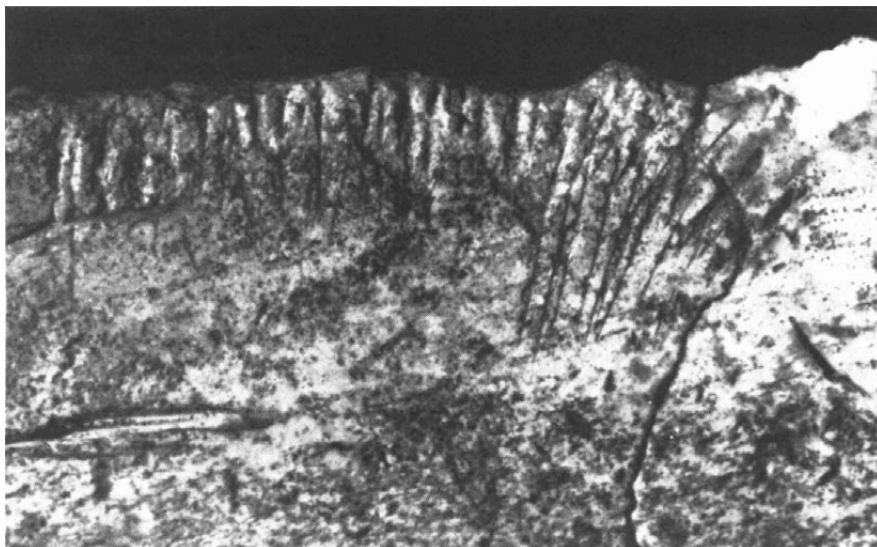


Fig. 2 Enlargement of the mane of the Sherborne horse. Photo: Jill Cook.

head that is very similar to that engraved on the Creswell Crags bone and rather dissimilar to the Sherborne variant. Sollas suggested that the illustration of the Creswell Crags horse, published (for the second time) by Boyd Dawkins in 1880, was the inspiration for the Sherborne piece, in that both show head and forequarters of a horse, facing right, with an upstanding mane. There seems some force in this argument, for the Sherborne horse is much more similar to Boyd Dawkins' rather poor illustration than to the original Creswell piece. Side by side the Sherborne and Creswell originals have little in common, the Creswell horse, for example is extremely delicately engraved, but this is not a characteristic that is conveyed in the illustrations and it is not a characteristic repeated in the Sherborne engraving. Furthermore, if the Sherborne copyist had seen the original Creswell engraving he might have appreciated the importance of the numerous additional odd lines that occur on the surface of this bone. Some of these appear in Boyd Dawkins' illustration, but might here well be dismissed as cracks, although they are a very consistent feature of Palaeolithic decoration. It is unusual to find a Palaeolithic engraving that is devoid, as is the Sherborne horse, of any extraneous lines, though these are not always included in illustrations of the material. Obviously there is no way of conclusively determining the age of an engraving as opposed to the age of its bone support, but such evidence as can be cited suggests that the Sherborne horse is not an authentic Palaeolithic engraving. The catalogue of Palaeolithic art pieces from this country is very small but to augment it by including the Sherborne horse appears to be quite unjustifiable. □

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Geology and health

from a Correspondent

THE influence of minerals and chemicals naturally occurring in the environment on health, together with the problems that arise in the mining and industrial use of minerals were reviewed at a recent meeting in London*. P. C. Elmes and J. C. Wagner (MRC Pneumoconiosis Unit) reviewed the diseases resulting from occupational and environmental exposure to mineral dusts. On the one hand are those which lead to lung scarring and malfunction such as silicosis, coal miners' pneumoconiosis and asbestosis, in which the severity of the symptoms is directly related to the intensity and length of exposure, and on the other are the more insidious cancers, where exposure may be small and the time lag to distress and diagnosis as much as 30–40 years. The load of dust in the lung associated with disease varies widely, from several hundred grams of coal dust, 5–10 g of pure silica to as little as 1 mg of asbestos. Both asbestos exposure and smoking increase the risk of lung cancer, but combining the two multiplies the risk. A recent discovery of an extremely high prevalence of the rare pleural mesothelioma in the village of Karain, in Cappadocia, Turkey, where in one year all 11 deaths out of 800 inhabitants were due to this one disease (there were no cases in the surrounding villages), has been linked with the presence of a zeolite mineral, erionite, which is found in the volcanic tuff rock from which local houses are built, and which weathers to form the soil on which this agricultural community depends. Similar fibrous minerals of 0.01–0.5 μm in diameter and 5–15 μm in length, a dimension that is retained in the body tissues and causes cell damage, occur in

other geological materials and must be a cause of concern.

The large-scale development of natural resources to meet the energy crisis will have important environmental consequences. W. Chappell (Center for Environmental Sciences, University of Colorado) forecast the production of 1 million barrels of oil a day from oil shale in the US by the late 1990s, requiring the processing of 500 million tons of shale per annum, as much as the present-day coal production in the US. Problems of waste disposal, release of toxic trace elements and organics into surface and ground waters and emissions of SO_2 into the atmosphere will expose workers and local communities to possible health hazards. Projected large scale expansion in surface coal mining over the next five years has led to studies linking epidemiology and water quality in the Midwestern USA, a programme funded by the Environmental Protection Agency and undertaken by Betsy Kagey (Downstate Medical Center, New York) and B.G. Wixson and N.L. Gale of the Environmental Research Center, University of Missouri — Rolla.

Geochemical investigations linking heavy metals in soils and the prevalence of dental caries were described by B.E. Davies (University of Wales, Aberystwyth) and R.J. Anderson (Dental School, University of Birmingham). Caries incidence has been found to be above normal where soils are heavily contaminated with lead from old metalliferous mining and smelting activities.

The Applied Geochemistry Research Group at Imperial College and the Institute of Geological Sciences have over the years mapped most of the country and geochemical atlases of England and Wales, Northern Ireland and parts of Scotland have been published. Research initiated by John Webb in the early 1960s has shown that regional geochemical differences can be successfully related to the health of crops and livestock, and that maps have uses in agriculture, water resources, pollution studies and public health. The Atlases will continue to provide unique sources of data on many different elements which can aid in selecting areas for food, water and medical surveys, particularly in rural parts of the country.

J. Lag (University of Oslo) reported that the Academies of Science in the five Scandinavian countries are setting up a committee to organise further cooperation in geomedical investigations. This will in part fulfil a similar role to the Working Party on Environmental Geochemistry and Health in Britain established in 1979 by the Royal Society under the chairmanship of S.H.U. Bowie. Consultation and cooperation in this expanding multidisciplinary research area should now be possible between scientists in the UK, Scandinavia, USA, Canada, USSR and Australia which now all have similar groups of interested scientists. □

*A one-day discussion meeting on Geology and Health was held on 30 January at the Geological Society of London, Burlington House, and organised by Dr Iain Thornton, Imperial College.

REVIEW ARTICLE

A list of interstellar molecules

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Interstellar molecules are useful guides to the conditions within interstellar clouds. The list presented here gives information on the sources, abundances and conditions of all interstellar molecules identified up until September 1979.

THE number of molecules identified in the interstellar medium has increased dramatically during the past decade: Fig. 1 shows the number discovered per year. As well as being of intrinsic interest, the molecules are useful tracers of the interstellar gas, and they can inform us of conditions within the interstellar clouds. The wide chemical variety present interesting problems of formation.

We present in Table 1 a list of interstellar molecules presently (September 1979) identified, and some information pertaining to them. The information selected is that likely to be of interest to a general reader and to those interested in the physics and chemistry of interstellar clouds, rather than in making observations. The first discovery is noted, and survey references are given, with some information regarding sources, abundances and conditions. An excellent compilation of radio and microwave frequencies of observed interstellar molecular transitions has been prepared by Lovas, Snyder and Johnson¹. We do not attempt to duplicate that information here.

For convenience, we have attempted to make Table 1 as concise as possible and have not included all lines observed for each molecule. In general, the information for each molecule is usually given on two lines of the table: the information on each line is from the reference on that line. The first line is devoted to the discovery reference, the second to a survey reference, if available. (Some particularly important molecules—H₂, OH and CO—are given extra space.) A description of Table 1 now follows; the molecules are arranged by number of atoms they contain, and then by molecular weight.

Column 1

Chemical formula, and name: the main isotopic version is given first; minor isotopes follow, in order of atomic weight. Atoms not identified by an isotopic weight are the predominant isotope: that is, C implies ¹²C.

Column 2

References: the first reference given is that of the first observation; the information on the same line in Table 1 refers to this reference. On the second line of information for the same molecule, another reference is given, where possible. This is a reference to a survey, if available.

Column 3

Sources. On the first line are given the sources in which molecule was first observed; on the second line the number of sources in the survey reference, or main sources in the second reference.

Column 4

Spectral line: this contains some information about the spectral lines observed. Ultraviolet (UV), optical (OPT), and infrared electronic (IR) transitions are indicated. Otherwise, the lines are in the microwave or radio spectral regions. The type of transition

is briefly indicated; electronic bands defined; 'Rot' implies a rotational transition; Λ implies that Λ -doubling is observed; and 'Hfs' implies that hyperfine structure is detected in the observations.

Column 5

Spectrum: absorption (A), emission (E), or maser emission (M) is indicated.

Column 6

Column density: the number given is the column density, per cm², calculated by the authors of the work referenced, under the assumptions listed in column 7. If column densities are not available, other relevant information, such as abundance ratios, may be given.

Column 7

Conditions: assumed (or inferred). Assumed information may involve the total gas number density n , per cm³, and temperature (such as rotation, spin, excitation, and kinetic) in degrees Kelvin. The type of calculation used by the authors is also mentioned. Inferred information about the source, if given, is in brackets.

Column 8

Comment: any useful remark is given here.

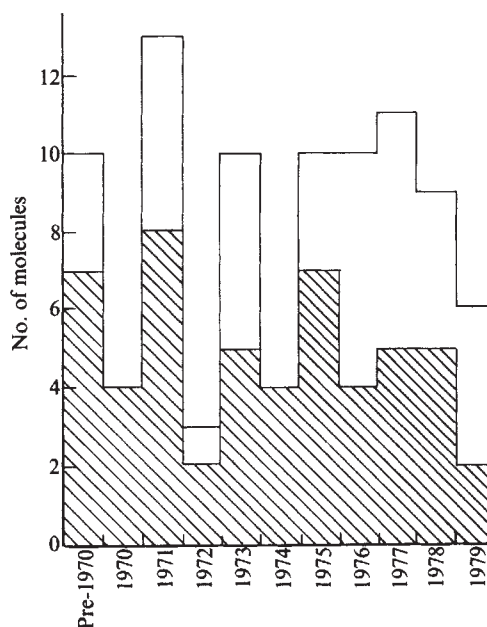


Fig. 1 Rate of discovery of interstellar molecules in recent years. Hatched totals ignore isotopic varieties.

Table 1 Interstellar molecules

Column 1	2	3	4	5	6	7	8
Molecule	Refs	Sources	Spectral line	Spectrum	Column density	Conditions assumed (or inferred)	Comment
Diatomics							
H ₂	6	ξ Per	UV: Lyman bands	A	~10 ²⁰	(T ₁₀ = 60)	a, Detected where E(B - V) ≥ 0.03
Molecular hydrogen	7	a	UV: Lyman bands	A	b	(T ₁₀ = 77 ± 17)	b, [H ₂] = [H] where E(B - V) ≥ 0.1
	8	BN, KL	IR 1-0 Vib-Rot	E	~10 ¹⁹	T ~ 2000	see also refs 9, 10
HD	11	9, inc. ξ Per	UV: Lyman bands	A	10 ¹⁴ -10 ¹⁵	T ₁₀ from	HD formed and destroyed more
Deuterium hydride	12	13 sources	UV: Lyman bands	A	<10 ¹⁵	H ₂ observations	rapidly than H ₂
CH ⁺	13	75 stars	OPT: A-X bands	A	≤10 ¹³	opt. thin	
Methylidyne ion	14	300 stars	OPT: A-X bands	A			see also ref. 15
¹³ CH ⁺	16	ζ Oph	OPT: A-X bands	A	~1/60[CH ⁺]	opt. thin	Negative results for ¹³ CH ⁺ at
CH	18	Many: a	OPT: ² Δ- ² Π bands	A	≤10 ¹³	—	ref. 17; ref. 16 single line only
Methylidyne	19, 162	Cas A	² Π _{1/2} , J = 3/2, A, Hfs	E	b	Non thermal	a, seen in 25% of sample 300
OH	23	Cas A	² Π _{3/2} , J = 5/2, A, Hfs	A	~2 × 10 ¹⁴	LTE, T = 10	OB stars, see ref. 14
Hydroxyl	26	Survey, 87 sources	—	M	—	—	b, Surveys (refs, 21, 22) N _{radio} ≤ 10 ¹⁴
	27	oPer	UV: D-X	A	~10 ¹³	—	1st MW emission lines, see ref. 24
¹⁷ OH	25	Sgr A	² Π _{3/2} , J = 3/2, A, Hfs	A	a	opt. thin	Many other MW lines detected
¹⁸ OH	29	Sgr A	² Π _{3/2} , J = 5/2, A, Hfs	A	~10 ¹²	opt. thin	Tentative; other UV observed in ref. 28
	30, 31	Sgr A, B2	² Π _{3/2} , J = 5/2, A, Hfs	A	—	opt. thin	a, [¹⁸ OH] = 4[¹⁷ OH]
C ₂	32	Cyg OB2 No. 12	IR Phillips bands	A	~10 ¹⁴	T _K = 35	Agrees with terrestrial ratio
Diatomic carbon	33	ζ Oph	IR Phillips bands	A	~8 × 10 ¹²	T = 22	[¹⁸ O]/[¹⁶ O] > 200
	34	ζ Oph	UV: D-X(0-0)R(0)	A	3 × 10 ¹²	T _K ≤ 16	
CN	18	Many: a	OPT: B-X bands	A	—	—	4σ detection
Cyanogen	35	Ori A, W51	1-0, Rot, Hfs	E	~10 ¹⁵	Boltzmann, T _R = 50	a: see in 25% of sample 300
CO	37	Ori A + 8 others	1-0, Rot	E	>10 ¹⁷	See ref. 38	OB stars, see ref. 14 Survey ref. 36
Carbon monoxide	39, 40	Surveys	1-0, Rot	E	10 ¹⁸ -10 ¹⁹	T = 10-50	V. abundant, found in V. many sources
	43	ζ Oph	UV: A-X Bands	A	10 ¹⁵	—	(2-1) Rot, see ref. 41; (3-2) Rot, see ref. 42
	45	3 sources	IR: 1-0 Vib Rot	A	~10 ²⁰	T = 650	see also ref. 44
¹³ CO	43	ζ Oph	UV A-X Bands	A	~10 ⁻² [CO]	CO opt. thick	Circumstellar. other bands see ref. 34
	47	W51; Sgr A, B2	1-0, Rot	E	—	—	See also refs 39 and 46
C ¹⁷ O	20	Ori A, ρ Oph	1-0, Rot	E	[C ¹⁸ O]/[C ¹⁷ O]	opt. thin	See ref. 45 for Vib-Rot lines
	48	9 sources	1-0, Rot	E	≈ 4	opt. thin	
C ¹⁸ O	47	W51; Sgr A, B2	1-0, Rot	E	—	opt. thin	
	39	8 sources	1-0, Rot	E	—	opt. thin	
¹³ C ¹⁸ O	39	NGC2024	1-0, Rot	E	v. weak	opt. thin	
NO	49	Sgr B2	² Π _{1/2} , J = 3/2 ← 5/2, A	E	2 × 10 ¹⁶	Thermal, T = 20	Also assumed N = 10 ²⁴
Nitric oxide	—	—	—	—	—	opt. thin	Apparently very rare in Galaxy
CS	50	Ori A, + 3 others	3-2, Rot	E	10 ¹³ -10 ¹⁵	opt. thin thermal	
Carbon monosulphide	51	30 sources	1-0,2-1, Rot	E	10 ¹³ -10 ¹⁵	T = 40, n = 10 ⁵	Only observed in dense regions
¹³ CS	50	Ori A, + 3 others	3-2, Rot	E	—	as CS	Deduced: [¹² C]/[¹³ C] ~ 90, (40 at Sgr)
	52	Sgr B2 + 3 others	1-2-3, Rot	E	—	—	
C ³³ S	51	Sgr B2	2-1, Rot, Hfs	E	—	opt. thin	Triplet splitting seen
	52	Sgr B, Ori A	3-2, Rot, Hfs	E	—	—	
C ³⁴ S	51	Ori A, + 4 others	2-1, Rot	E	—	opt. thin	
	52	Sgr B, + 3 others	3-2, Rot	E	—	—	
SiO	53	Sgr B2	3-2, Rot, v" = 0	E	~4 × 10 ¹³	Thermal T = 50	[¹³ CS]/[¹² CS] ≈ 0.4-0.6
Silicon monoxide	54	16 sources	1-0, Rot, v" = 1	E, (M)	—	opt. thin	Usually in maser emission near
²⁹ SiO	56	Ori A	2-1, Rot, v" = 0	E	~0.05 [SiO]	opt. thin	stars and H II regions. See also ref. 55
	—	—	—	—	—	—	Deduces SiO not masering in
³⁰ SiO	57	Ori A, Sgr B2	2-1, Rot, v" = 0	E	—	opt. thin	Ori A
	—	—	—	—	—	—	Not masering
SO	58	Ori A + 6 others	4 ₃ -3 ₂ -2 ₁ , Rot	E	≈ 2 × 10 ¹⁴	See ref. 69	
Sulphur monoxide	59	18 sources	5 Rot transitions	E	≈ 2 × 10 ¹⁴	—	Kleinman-Low Neb., N(SO) ~ 10 ¹⁶
³⁴ SO	59	Ori A, KL	3 ₂ -2 ₁ , 2 ₃ -1 ₂ , Rot	E	a	opt. thin	a, consistent with terrestrial
	—	—	—	—	—	—	³⁴ S/ ³² S
NS	60	Sgr B2	² Π _{1/2} , J = 3/2 ← 5/2, A, Hfs	E	~10 ¹⁴	T = 20, n = 10 ⁶	
Nitrogen sulphide	61	Sgr B2	² Π _{1/2} , J = 3/2 ← 5/2, A, Hfs	E	?	Stat. eqm.	Anomalous excitation?
SiS	62	Sgr B2, IRC + 10216	6-5-4 Rot	E	4 × 10 ¹³	T = 50. opt. thin	Sgr B2: 6-5 only. Tentative?
Silicon sulphide	—	—	—	—	—	—	
Triatomics							
H ₂ O	63	Sgr B2, + 2 others	6 ₁₆ -5 ₂₃ Rot	E, M	> 10 ¹⁷	?	High excitation (465 cm ⁻¹ above
Water vapour	64, 161	9, 10 sources	6 ₁₆ -5 ₂₃ Rot	M	—	—	ground)
HDO	65	Ori A, KL	1 ₁₀ -1 ₁₁ , Rot	E, M	10 ¹⁶ -10 ¹⁷	T = 70, n = 10 ⁷	Surveys: all maser sources
	—	—	—	—	—	—	Not as strongly masering as
H ₂ ¹⁸ O	66	Ori A, KL, DR21	3 ₂₀ -3 ₁₃ (para), Rot	E, M	~4 × 10 ⁻³ cm ⁻³	T ~ 50, n = 10 ⁶	H ₂ O
	—	—	—	—	—	—	Weak maser. Assuming
C ₂ H	67	13 sources	1-0, Rot, Hfs	E	5 × 10 ¹⁴	T = 100	terrestrial ¹⁶ O/ ¹⁸ O, then
Ethynyl	—	—	—	—	~3 × 10 ¹⁵	—	n(H ₂ O) = 8 cm ⁻³
	—	—	—	—	—	—	Upper limit: levels thermalised
HCN	68	6 sources	1-0, Rot, Hfs	E	~10 ¹⁵	Boltzmann, T = 20	Lower limit: lowest two levels
Hydrogen cyanide	69	4 sources	1-0, Rot, Hfs	E	a	—	only
DCN	70	Ori A	2-1-0, Rot, Hfs	E	~4 × 10 ¹²	Thermal, T = 10	a, extensive modelling of line
	71	7 sources	2-1, Rot, Hfs	E	—	—	width, n _T , T
H ¹³ CN	68	Ori A, Sgr B2	1-0, Rot, Hfs	E	[DCN]/[H ¹³ CN]	—	a, DCN/HCN usually high
	—	—	—	—	variable	—	(lower in Sgr B2)
	71	14 sources	1-0, Rot, Hfs	E	—	—	
HC ¹⁵ N	72	Ori A	2-1, Rot	E	—	opt. thin	Other refs: 73 and 69
	73	6 sources	—	—	—	—	[HC ¹⁵ N]/[H ¹³ CN] ≈ 2.6
HNC	75	—	1-0, Rot	E	T _K uncertain	—	Another survey in ref. 74
	—	—	—	—	—	—	ref. 76 is lab. confirmation of
Hydrogen isocyanide	77	OriA, + 3 others	—	—	—	—	microwave spectrum
DNC	77	OriA, + 3 others	2-1, Rot	E	[HNC]/[DNC]	a	a, abundance of DNC higher in
	78	NGC2264	1-0, Rot	E	variable	—	cooler clouds?
HN ¹³ C	79	5 sources	1-0, Rot	E	—	—	
	—	—	—	—	—	—	
H ¹⁵ NC	80	DR(21)OH	1-0, Rot	E	[H ¹⁵ NC]	opt. thin	Enrichment of ¹⁵ N(?)
	—	—	—	—	~[HN ¹³ C]	—	
HCO ⁺	81	—	1-0, Rot	E	—	a	Predicted: (ref. 82); lab. confirma-
	—	—	—	—	—	—	tion: ref. 83
Formyl ion	84, 85	Surveys	—	—	—	—	a, theory predicts HCO ⁺ most
	—	—	—	—	—	—	abundant ion

Table 1—continued

Column 1	2	3	4	5	6	7	8
Molecule	Refs	Sources	Spectral line	Spectrum	Column density	Conditions assumed (or inferred)	Comment
DCO ⁺	86	3 sources	1-0, Rot	<i>E</i>	[H ¹³ CO ⁺]/[HCO ⁺] [DCO ⁺]	opt. thin	High [DCO ⁺]/[HCO ⁺] implies $X(e) < 10^{-8}$ in dense clouds
H ¹³ CO ⁺	87	8 sources	1-0, Rot	<i>E</i>	$\approx 0.1[\text{HCO}^+]$	opt. thin	Confirms HCO ⁺ detection
HC ¹⁸ O ⁺	89	L134, TMC1	1-0, Rot	<i>E</i>		opt. thin	See also ref. 86
HCO	90	Sgr B2	1-0, Rot	<i>E</i>		opt. thin	[H ¹³ CO ⁺]/[HC ¹⁸ O ⁺] ~ 6
	91	4 sources	$1_{01}-0_{00}$, $J = \frac{3}{2}-\frac{1}{2}$, $F = 2-1$	<i>E</i>	$\sim 10^{13}$	$T = 20$	$\sim$ terrestrial isotope ratios [HCO] < [HCO ⁺]
Formyl N ₂ H ⁺	—	—	—	—	—	—	—
N ₂ D ⁺	92	11 sources	1-0, Rot, Hfs	<i>E</i>	$\sim 3 \times 10^{13}$	$T_s = 10$	ref. 93: calculation, and ref. 94: lab. spectrum
H ₂ S	95	L134N	1-0, Rot, Hfs	<i>E</i>	$\sim 10^{12}$	opt. thin. $T_K \approx 15$	N ₂ H ⁺ /N ₂ D ⁺ ≈ 2 . Enormous isotope fractionation
Hydrogen sulphide	96	7 sources	$1_{10}-1_{01}$ Rot	<i>E</i>	$\approx 10^{15}$	opt. thin $T = 20$	
HNO	97	Sgr B2, NGC2024	$1_{01}-0_{00}$ Rot	<i>E</i>	$\approx 10^{15}$ (Sgr B2)	opt. thin Boltzmann $T < 100$	$N \approx 10^{14}$ (NGC2024) tentative?
Nitroxyl	—	—	—	—	—	—	—
OCS	98	Sgr B2	9-8 Rot	<i>E</i>	$\approx 3 \times 10^{15}$	opt. thin	See also ref. 100
Carbonyl sulphide	99	Several, Rot	Several, Rot	<i>E</i>		Boltzmann $T < 50$	Narrow (interstellar?) and broad (circumstellar?) com- ponents seen
SO ₂	101	Ori A, Sgr B2	Several, Rot	<i>E</i>	$\sim 10^{16}$	opt. thin	
Sulphur dioxide	—	—	—	—	—	Boltzmann $T \approx 100$	
4-atomics							
NH ₃	102	Sgr A	(1,1)(2,2) inversion	<i>E</i>	$\sim 2 \times 10^{16}$	opt. thin	Seen in many dense clouds, possibly 'clumpy'?
Ammonia	106	11 sources	(1,1)(2,2) inversion	<i>E</i>			
NH ₂ D	103	Sgr B2	$1_{11}-1_{01}$ Rot	<i>E</i>	$\sim 10^{14}$	$n \sim 10^6$, opt. thin	[NH ₂ D]/[NH ₃] ~ 0.017
	104	Orion, KL	$1_{11}-1_{01}$ Rot	<i>E</i>	$\sim 10^{14}$	$50 \leq T_K \leq 150$	
¹⁵ NH ₃	105	Orion	(1,1)(2,2)(3,3)	<i>E</i>	<i>a</i>	opt. thin $T_R \sim 50$	a , [¹⁴ N]/[¹⁵ N] $\approx$ terrestrial
C ₂ H ₂	107	IRC+10216	Several, Rot	<i>A</i>	$\approx 10^{17}$	LTE $T = 300$	Circumstellar source, not 'genuine' interstellar
Acetylene	—	—	—	—	—	—	—
H ₂ CO	108	17 sources	$1_{11}-1_{10}$ Rot	<i>A</i>	$\sim 10^{14}$ (Sgr A)	<i>a</i>	<i>a</i> , $1_{10}-1_{10}$ is seen in absorption against 2.7 K BB
Formaldehyde	110	28 sources	—	<i>A</i>			
HDCO	109	L134N, CLD2	$2_{02}-1_{01}$ Rot	<i>E</i>	<i>a</i>	$T_{\text{kin}} = 10$	[HDCO]/[H ₂ CO] ≈ 0.1 D enhanced
H ₂ ¹³ CO	111	Sgr A, B2, W51	$1_{11}-1_{10}$ Rot	<i>A</i>	<i>a</i>	opt. thin	<i>a</i> , [H ₂ CO]/[H ₂ ¹³ CO] ~ 10 in galactic centre. W51 tentative
H ₂ C ¹⁸ O	112	Sgr B2	—	—	—	—	Using ref. 111
	113	Sgr B2	$1_{11}-1_{10}$ Rot	<i>A</i>	(¹³ C: ¹² C)/ (¹⁸ O: ¹⁶ O) ≈ 10	opt. thin	
	114	Sgr A, B2, W33	$1_{11}-1_{10}$ Rot	<i>A</i>	<i>b</i>	opt. thin	<i>b</i> , [H ₂ ¹³ CO]/[H ₂ C ¹⁸ O] ≈ 7
HNCO	116	Sgr B2, W51	$4_{04}-3_{03}$ Rot	<i>E</i>	$\sim 5 \times 10^{14}$	opt. thin	Also $1_{01}-0_{00}$ detected, ref. 115 First report ref. 75
Isocyanic acid	—	—	—	—	—	—	See also ref. 59
H ₂ CS	117	Sgr B2	$2_{12}-2_{14}$ Rot	<i>E</i>	$> 10^{16}$	opt. thin	Non thermal
Thioformaldehyde	118	Sgr B2	$4_{14}-4_{13}$ Rot	<i>A</i>	?		Based on calculated spectrum, ref. 120
C ₃ N	119	IRC+10216	$10-9-8$ Rot	<i>E</i>	$\approx 10^{15}$		
Cyanoethynyl	—	—	—	—	—	—	—
HNCS	163	Sgr B2	Rot, 3 lines	<i>E</i>	$\sim 2 \times 10^{13}$	$T_R = 14 \pm 5$, opt. thin	
Isothiocyanic acid	—	—	—	—	—	—	—
5-atomics							
CH ₄	122	Ori A	6 lines, Rot	<i>E</i>	$\sim 10^{22}$ (?)	Non thermal excitation	Column density uncertain
Methane	—	—	—	—	—	—	Tentative identification
CH ₂ NH	123	Sgr B2	$1_{11}-1_{10}$ (Mult) Rot	<i>E</i>	$> 3 \times 10^{14}$	opt. thin. $T_x \gg 25$	
Methanimine	—	—	—	—	—	—	—
CH ₂ CO	124	Sgr B2	Several, Rot	<i>E</i>	10^{14}	Stat. eqm.	Tentative identification of 3 more Rot. lines
Ketene	—	—	—	—	—	—	—
NH ₂ CN	125	Sgr B2	$4_{13}-3_{12}$, $5_{14}-4_{13}$ Rot	<i>E</i>	2×10^{13} -6×10^{14}	$n > 10^6$, $T_k \approx 20$ Stat. eqm. $n \leq 10^6$, $T_k \leq 60$	
Cyanamide	—	—	—	—	—	—	—
HCOOH	126	Sgr B2	$1_{11}-1_{10}$ Rot	<i>E</i>	$10^{12}-10^{13}$		Tentative? See ref. 5
Formic acid	127	Sgr B2	$2_{11}-2_{12}$ Rot	<i>E</i>	$\sim 5 \times 10^{13}$	$T_R = 2.2$	
C ₄ H	128	IRC+10216	$12-11-10-9-8$ Rot	<i>E</i>	$4 \times 10^{14}-3 \times 10^{15}$	$60 < T_{\text{ex}} < 600$	Detection based on theoretical calculation of rotational spectrum
Butadiynyl	—	—	—	—	—	—	—
HC ₃ N	129	Sgr B2	0-1 Rot	<i>E</i>	<i>a</i> : $\sim 10^{14}$	opt. thin. $T = 50$	<i>a</i> , corrected, see ref. 130
Cyanoacetylene	131	17 sources	7 transitions	<i>E</i>	$> 10^{14}$	Stat. eqm.	See ref. 131 for other refs
H ¹³ CCCN	132	Sgr B2	1-0, Rot	<i>E</i>	$\sim 0.05[\text{HC}_3\text{N}]$	opt. thin	
	133	Sgr B2, Ori A	9-8 Rot	<i>E</i>			¹² C/ ¹³ C = 22 (Sgr B2), 50 (Ori A)
HC ¹³ CCN	134	Sgr B2	0-1 Rot	<i>E</i>			
	133	Sgr B2, Ori A	9-8 Rot	<i>E</i>			
HCC ¹³ CN	134	Sgr B2	0-1 Rot	<i>E</i>			
	133	Sgr B2, Ori A	9-8 Rot	<i>E</i>			
6-atomics							
CH ₃ OH	135	SgrA, B2	$1_{10}-1_{11}$ Rot	<i>E</i>		opt. thin	Column densities higher towards Sgr and KL nebula
Methyl alcohol	136	14 sources	2-1, several, Rot	<i>E</i>	$\sim 6 \times 10^{14}$	Stat. eqm.	Other transitions ref. 88
¹³ CH ₃ OH	136	Sgr B2	2-1, several, Rot	<i>E</i>		Stat. eqm.	[¹³ CH ₃ OH]/[CH ₃ OH] ≈ 0.1
CH ₃ OD	136	Sgr B2	2-1, several, Rot	<i>E</i>		Stat. eqm.	[CH ₃ OD]/[¹³ CH ₃ OH] ≈ 0.1
CH ₃ CN	137	Sgr A, B2	6-5 Rot	<i>E</i>	2×10^{14}	Stat. eqm.	
Methyl cyanide	138	Ori A	5-4 Rot	<i>E</i>		$n > 10^6$, $T > 150$	
NH ₂ CHO	139	Sgr A, B2	$2_{11}-2_{12}$ Rot, Hfs	<i>E</i>	6×10^{14}	Stat. Eqm	
Formamide	140	Sgr A, B2	$1_{11}-1_{10}$ Rot, Hfs	<i>E</i>	$\sim 10^{15}$	$T = 100$, $n = 10^5$	
CH ₃ SH	121	Sgr B2	Rot, 6 lines	<i>E</i>	$\sim 10^{14}$	$T_R = 9 \pm 3$, opt. thin	
Methylmercaptan	—	—	—	—	—	—	—

Table 1—continued

Column 1	2	3	4	5	6	7	8
Molecule	Refs	Sources	Spectral line	Spectrum	Column density	Conditions assumed (or inferred)	Comment
7-atomics							
CH ₃ NH ₂	141	Sgr B2, Ori A	5 ₁₅ -5 ₀₅ , 4 ₁₄ -4 ₀₄ , Rot	E	~10 ¹⁵	Boltzmann	
Methylamine	142	Ori A	2 ₀₂ -1 ₁₀ , Rot	E	~10 ¹⁵	T _x = 40	
CH ₃ NHD	143	Sgr A, B2	2 ₀₂ ⁽⁺⁾ -1 ₁₀ ⁽⁻⁾ Rot	E, A		30 ≤ T _x ≤ 60	Tentative Detection, not confirmed by independent search ref. 144
	—	Ori A, W51					
CH ₃ C ₂ H	145	Sgr B2	5 ₀ -4 ₀ , k = 1-4, Rot	E	~3 × 10 ¹⁵	opt. thin. Boltzmann T _R = 31, n ≥ 10 ⁴	
Methylacetylene	138	Sgr B2	5 ₀ -4 ₀ , k = 1-4, Rot	E		opt. thin	
CH ₃ CHO	146	Sgr A, B2	1 ₁₀ -1 ₁₁ , Rot	E	> 3 × 10 ¹⁴		Maser? (ref. 147)
Acetaldehyde	147	Sgr B2	2 ₁₁ -2 ₁₂	E			See also ref. 138
CH ₂ CHCN	148	Sgr B2	2 ₁₁ -2 ₁₂ Hfs Rot	E	~4 × 10 ¹³		Maser
Vinyl cyanide	149	Sgr B2	10 _{0,10} -9 _{0,9} , Rot	E	~3 × 10 ¹³		ref. 150: 2-1 line detection
HC ₃ N	151	Sgr B2	4-3 Rot	E	~10 ¹³	opt. thin. Boltzmann T = 30	Also ref. 152. Weak maser.
Cyanodiacetylene	153	TMC 1, 2	9-8 Rot	E	~10 ¹⁴		
8-atomics							
HCOOCH ₃	154	Sgr B2	1 ₁₁ -1 ₁₀ Rot	E	~2 × 10 ¹³	1% inversion	Tentative
Methyl Formate	—						
9-atomics							
CH ₃ CH ₂ OH	155	Sgr B2	Three lines, Rot	E	~10 ¹⁵	Stat. Eqm. T = 20, n = 10 ⁴ -10 ⁵	
Ethyl alcohol	156	Ori A	6 ₀₆ -5 ₁₅ Rot	E	~10 ¹⁵	Boltzmann T = 10-100	Also: 2 ₂₀ -2 ₁₁ -2 ₀₂ tentatively identified
(CH ₃) ₂ O	—						
Dimethylether	—						
CH ₃ CH ₂ CN	157	Ori A, Sgr B2	24 lines OriA } 7 lines Sgr B2 }	Rot E	~10 ¹⁴	LTE T = 20 opt. thin	
Ethyl cyanide	158	Heiles 2 = TMC1	9-8, 21-20, Rot	E	~3 × 10 ¹³	Stat. Eqm. T = 8, n = 3 × 10 ³	Similar results assuming LTE
HC ₇ N	158						
Cyanotriacetylene (or cyanoheptatriyne)	159	TMC 1, 2	22-21, Rot	E	~2 × 10 ¹²		
11-atomics							
HC ₉ N	160	Heiles 2	18-17, 25-24, Rot	E	~3 × 10 ¹²	opt. thin T = 11, n = 4 × 10 ⁴ Coll. Ex.	Rot. spectrum extrapolated from cyanopolylene series
Cyano-octatetra-yne							

Discussion

We have occasionally indicated that a detection may be only tentative. Factors tending to confirm the presence of a molecule are: (1) the existence of several spectral lines, all showing the same velocity shift; (2) detection in a number of astronomical sources; (3) the identification of an isotopic version of the molecule at the corresponding frequencies; and (4) hyperfine structure at the correct splitting. Thus, for example, HCO⁺ observed in but a single line, is confirmed not only by the laboratory spectrum, but also by the isotopic versions H¹³CO⁺ and DCO⁺, at the correct frequencies, in the same astronomical sources.

Some ambiguity occurs: when is a molecule circumstellar, and when interstellar? Clearly no nice division occurs, and we have not attempted to make one. The source Sgr B2 towards the

galactic centre remains the richest, but there are some surprises here; it does not exhibit (as yet) the three most massive molecules known. Isotopic ratios are important indicators of interstellar chemistry, and, again, the galactic centre seems to be an anomalous region in this respect. Fractionation of isotopes is apparently very effective; certain isotopic versions may be overabundant by orders of magnitude.

Of these 90 molecules, the chemistry of, perhaps, 10 is thought to be understood, though theoretical models are often in conflict with observation. The list is a reminder of the complexity of the problems awaiting solution. The interested reader will find the astrophysical context well reviewed in refs 2-5.

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ARTICLES

Interstellar extinction in the Large Magellanic Cloud

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Recent UV observations together with complementary visible data of several reddened and comparison stars of similar spectral types in the Large Magellanic Cloud have been used to study the interstellar extinction in that galaxy. Most of the reddened stars studied here are located within 2° of 30 Doradus and show remarkably high extinction in the far UV, suggesting a large abundance of small particles. From the optical wavelength to 2,600 Å the normalised extinction curves of the LMC stars are similar to the mean galactic extinction law.

THE Large Magellanic Cloud (LMC) is the nearest extragalactic system and a preliminary study of the UV spectra of LMC members shows the importance of the IUE¹ for stellar and interstellar work in the Magellanic Clouds. The Clouds contain dust though to a lesser extent than the Galaxy. A recent survey of the chemical composition of H II regions in the Magellanic Clouds² reveals that oxygen is deficient in these galaxies by about a factor of four relative to the Orion nebula. The composition of the interstellar dust and gas in the Clouds provides important information on the origin of the dust and

the chemical enrichment of the interstellar media in these galaxies.

The wavelength dependence of interstellar extinction over a wide range of wavelengths from the visible to the UV depends on the composition and relative abundances of the agents responsible for scattering and absorption³. The characteristic feature of the galactic extinction curve is the broad symmetric absorption band ($\Delta\lambda \sim 750$ Å) centred near 2,200 Å (ref. 4). Earlier attempts to determine the UV extinction law in the LMC, using surface brightness measurements obtained with the

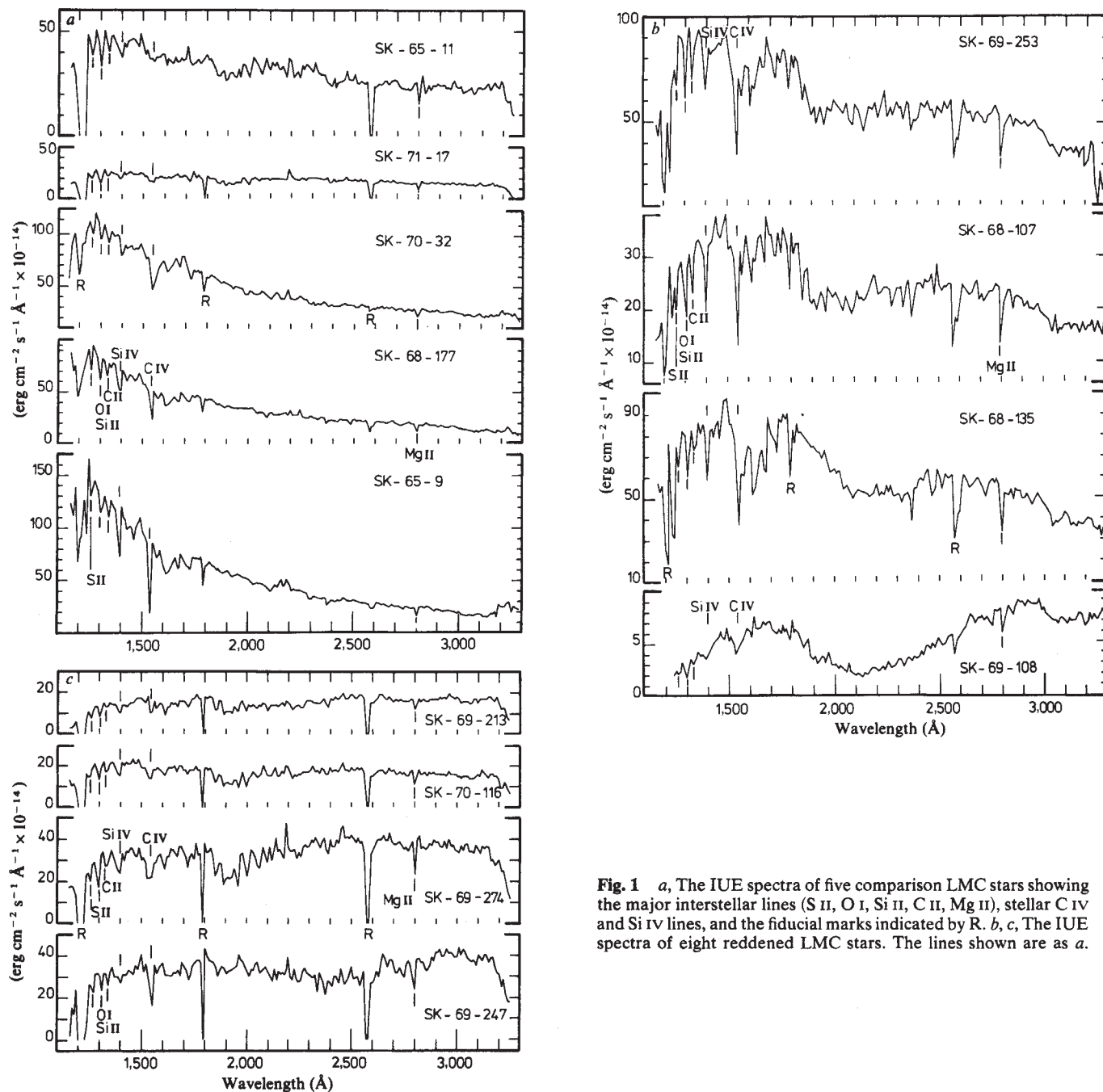


Fig. 1 *a*, The IUE spectra of five comparison LMC stars showing the major interstellar lines (Si II, O I, Si II, C II, Mg II), stellar C IV and Si IV lines, and the fiducial marks indicated by R. *b*, *c*, The IUE spectra of eight reddened LMC stars. The lines shown are as *a*.

S2/68 (ref. 5) and ANS experiments^{6,7} have led to discrepant results. The detection of the 2,200 Å feature in the spectrum of a reddened LMC member, SK-69-108, has recently been reported⁸. This article presents further observations, in both the visible and the UV, of a larger sample of LMC stars to study interstellar extinction in the LMC.

Observations

The IUE^{9,10} controlled from the ESA satellite tracking station at Villafranca, Spain, was used in its low resolution mode ($\Delta\lambda \sim 6$ Å) to obtain UV spectra of the reddened and comparison stars. Complementary visible spectra which extend from 3,980 to 6,140 Å were obtained at a dispersion of 110 Å mm⁻¹ using the image dissector scanner at the Cassegrain focus of the 3.6 m telescope of the ESO at La Silla, Chile. Table 1 lists the reddened and comparison stars observed, together with their coordinates, photometric data and spectral types¹¹⁻¹³.

The reduced UV spectra provided before August 1979 by the IUE ESA Observatory contained ITF errors in the short

wavelength channel. Consequently, the photometric correction of the raw images of these spectra was repeated with the correct intensity transfer functions (ITF) using a program developed by J. Fettle (personal communication), and the corrected spectra extracted. The UV spectra in the long wavelength channel were provided by the ESA Observatory. Absolute fluxes were obtained using the adopted sensitivity calibration curve¹⁴.

The wavelength scales were checked by examining the positions of the principal interstellar lines: 1,260 Å (blend of Si II and S II), 1,303 Å (O I, Si II), 1,335 Å (C II), 2,342 Å (Fe II), 2,382 Å (Fe II), 2,598 Å (Fe II) and 2,798 Å (Mg II). The wavelength scales of the reduced spectra were found in all cases to be accurate to ± 4 Å. The raw images were checked for fiducial marks, noise, spikes and saturation effects. Data affected by saturation and fiducial marks were excluded. Except for the short wavelength spectrum of SK-69-213, all spectra were well exposed with average signal over all spectral ranges of interest lying over 100 DN (IUE data number). The UV flux distributions in absolute units smoothed over 10 Å bands of the comparison and reddened stars are shown in Fig. 1a-c.

The visible spectra were obtained on one night (25 December 1978). The wavelength and flat field calibration of the data, recorded on magnetic tape, was performed at ESO, Geneva, using He-Ne and flat field spectra taken immediately before the stellar observations. As the difference between the air masses at the beginning and the end of the observing night was small, the visible spectra were corrected for atmospheric extinction using the mean law appropriate for La Silla (by Dr H. Pederson of the ESO). The visible spectra were reduced to give data points every 1.5 Å and in the subsequent analysis average counts in 60 Å wavelength intervals from $\lambda^{-1} = 1.66$ to $2.54 \mu\text{m}^{-1}$ were utilised. The photometric accuracy of the average counts is $\sim \pm 3\%$.

Measurement of interstellar extinction

The analysis of the spectral features in the UV and visible data obtained will be given elsewhere. Note, however, that the reddened stars listed in Table 1 clearly show the diffuse interstellar feature at 4,430 Å similar to that observed in reddened stars in the galaxy. The equivalent width of this feature is comparable with that computed from the colour-excess, $E(B - V)$, from the mean galactic relationship between W_{4430} and $E(B - V)$ (ref. 15).

To measure the extinction in the visible wavelength range, a set of extinction curves was derived for each of the reddened stars using comparison stars, and a mean extinction curve was determined for each reddened star, thus reducing the errors introduced by possible spectral type mismatch. The extinction in magnitudes, $\Delta m(\lambda)$ has been normalised to $\Delta m = 0$ at 5,500 Å (the effective wavelength of the *V*-band) and $\Delta m = 1$ at 4,300 Å (the effective wavelength of the *B*-band). Within observational errors, the individual extinction curves in the visible agree well thereby allowing a mean extinction curve to be constructed. The mean curve was computed in the wavelength range, $\lambda^{-1} = 1.66$ – $2.94 \mu\text{m}^{-1}$ with r.m.s. error $< \pm 0.3$ mag, and extended to the *U*-band using the available *UBV* photometric data. This mean curve seems to be identical to the mean galactic extinction law in the visible as derived by Seaton¹⁶.

For the measurement of interstellar extinction in the UV, the comparison stars SK-67-108 and SK-67-111, which were observed with the small (3×3 arcs) IUE spectrograph slits, were excluded. To improve the photometric accuracy of the UV data, the stellar fluxes were averaged in 50 Å bands from 1,150 to 3,000 Å. The mean photometric accuracy of a single observation has been estimated from the difference in the colours ($m_\lambda - m_{2,900\text{Å}}$) of stars with more than one observation: it is

$\sim \pm 0.05$ mag except near the extremities of the wavelength channels where it is much larger, about ± 0.3 mag.

The UV extinction has been measured for pairs of reddened and comparison stars which match in spectral type. The extinction, Δm is normalised to $\Delta V = 0$ and $\Delta E(B - V) = 1$, where ΔV is the difference in the *V* magnitudes and $\Delta E(B - V)$ is the difference in the colour-excesses of the reddened and comparison stars. The UV extinction curves for eight reddened stars are shown in Fig. 2; the ordinate is the normalised extinction,

$$\Delta m = \frac{A_\lambda - A_V}{E(B - V)}$$

Discussion

Apart from photometric errors, the major source of error in the extinction measurements constructed from the pairs of reddened and comparison stars is the mismatch in spectral type and luminosity class. For the sample of LMC stars studied here, the spectral type error is estimated to be $\sim \pm 1$ subclass. This estimate is based on an examination of the important spectral features both in the visible and in the UV. However, the difference in absolute magnitude, ΔM_V , between the reddened and comparison stars is ~ -1.6 mag. Galactic supergiants have a flux deficiency in the far UV (shortward of 1,600 Å) as compared with giants and dwarfs¹⁷. However, from a study of the spectra of galactic stars which appear in the Ultraviolet Bright-Star Catalogue¹⁸ it is found that the UV flux distributions of B0-B3Ia stars do not differ significantly from those of B0-B3Ib stars, the differences being well within the photometric errors. The error in the measured extinction due to spectral type mismatch of ± 1 subclasses per unit normalising factor is $\sim \pm 0.2$ mag near 2,740 Å increasing to $\sim \pm 0.4$ mag at 1,400 Å.

The interesting feature of Fig. 2 is that shortward of $\lambda^{-1} = 3.8 \mu\text{m}^{-1}$ the extinction curve for SK-69-108 is clearly different from those of the other stars: shortward of $5.5 \mu\text{m}^{-1}$ the extinction for SK-69-108 is on average 2 mag lower than that for the other stars and near 2,200 Å ($4.6 \mu\text{m}^{-1}$) it is ~ 1 mag greater. But longward of 2,600 Å ($\lambda^{-1} = 3.8 \mu\text{m}^{-1}$), the normalised extinction values for all the stars are the same. Note that the reddened star SK-69-108 is situated in the bar, while the other reddened stars are located to the north-east of the bar within 2° of the 30 Doradus region, a site of recent star formation. This raises the interesting possibility that the extinction properties of the dust in the latter region may differ from those of the dust in

Table 1 Programme stars observed in the LMC

Reddened stars							
Star	RA (1975)	Dec (1975)	Spectral type	<i>V</i>	<i>B - V</i>	<i>U - B</i>	Spectral range observed
SK-68-107	5h 30.8 min	-68° 26'	B0Ia	12.22	0.03	-0.82	Visible to UV
SK-68-135	5 38.0	-68 56	O9.5I	11.36	0.00	-0.86	Visible to UV
SK-69-108	5 20.1	-69 54	B3I	12.10	0.27	-0.49	Visible to UV
SK-69-213	5 36.4	-69 12	B1	11.97	0.10	-0.75	UV
SK-69-253	5 39.7	-69 28	B0I	11.23	-0.02	-0.81	Visible to UV
SK-69-247	5 39.1	-69 31	B6I	10.42	0.17	-0.54	UV
SK-69-274	5 41.7	-69 49	B2.5Ia	11.21	0.04	-0.74	UV
SK-70-116	5 49.3	-70 3	B2Ia	12.05	0.11	-0.72	UV
Comparison stars							
SK-65-9	4 59.2	-65 51	B1	12.72	-0.22	-1.07	UV
SK-65-11	4 59.3	-65 45	B5I	11.66	-0.04	-0.69	UV
SK-67-108*	5 26.5	-67 38	B0	12.56	-0.20	-1.05	Visible to UV
SK-67-110	5 26.9	-67 29	B1:	11.62	-0.07	-0.94	Visible
SK-67-111*	5 26.9	-67 30	O8	12.57	-0.20	-1.03	Visible to UV
SK-68-177	5 53.0	-68 14	B2	12.93	-0.18	-0.96	UV
SK-70-32	5 0.5	-70 13	B0I	13.10	-0.21	-1.01	UV
SK-71-17	5 22.0	-71 58	B3Ia (ref. 13)	12.29	-0.06		UV
SK-69-249	5 39.2	-69 30	B0.5	(10.6)			Visible

* IUE observations recorded using the small spectrograph aperture. All other IUE data were obtained using the large aperture. Figure in brackets is photographic *V*-mag (ref. 11).

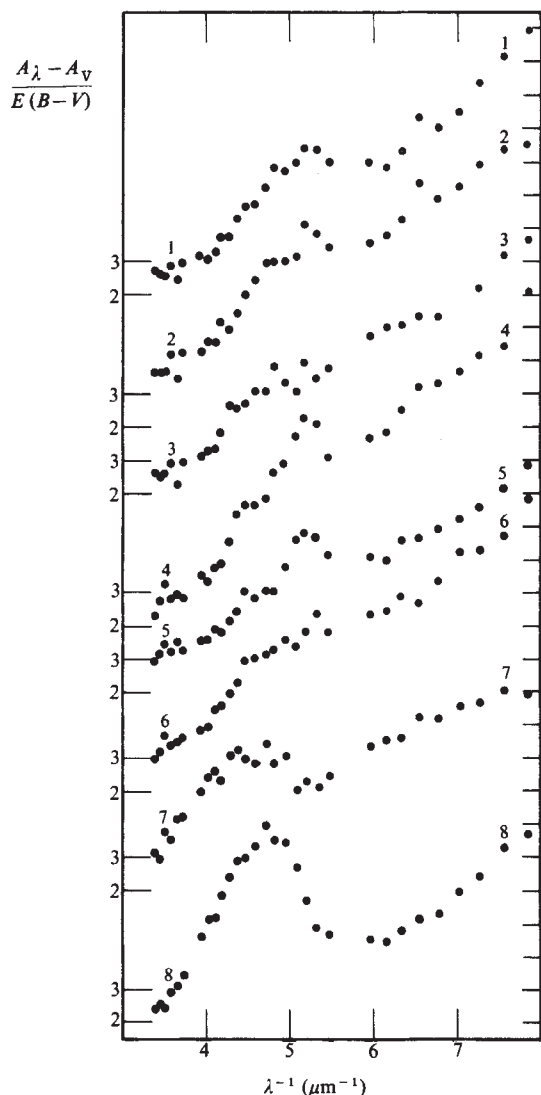


Fig. 2 Individual extinction curves normalised to $V=0$ and $E(B-V)=1$ derived from IUE observations. The numbers 1–8 denote the following pairs of reddened and comparison stars: 1, SK-68-107, SK-70-32; 2, SK-69-253, SK-70-32; 3, SK-68-135, SK-70-32; 4, SK-69-274, SK-68-177 and SK-71-17; 5, SK-70-116, SK-68-177; 6, SK-69-213, SK-65-9; 7, SK-69-274, SK-65-11; 8, SK-69-108, SK-71-17.

the bar. As the extinction curves of seven of the stars have similar shapes, they have been combined to give a mean curve. This curve is shown in Fig. 3 with the extinction curve for SK-69-108; also shown in Fig. 3 are the mean visible extinction curve as determined for the LMC stars, and for comparison the galactic extinction law of Seaton¹⁶. The mean normalising factor being 4, the r.m.s. error bar shown in the mean extinction curve can be explained in terms of the observational errors. Within this error, the extinction curve for SK-69-108 is close to the galactic law, while the mean extinction curve for other stars shows an increase in extinction by ~ 3 mag in the far UV shortward of $\lambda^{-1} = 6 \mu\text{m}^{-1}$, which is much larger than twice the r.m.s. error.

The $2,200 \text{ \AA}$ feature for the mean curve seems to be weaker than that for the galactic law, but not as much as suggested by Koornneef⁷. For example, the colour-excess ratio $E(2,200-2,500)/E(B-V)$ has a mean value of 2 for the seven stars, (2.7 for SK-69-108) and 2.5 for galactic stars. Between 4.6 and $5.5 \mu\text{m}^{-1}$ the mean extinction curve does not decrease as much as it does in the case of SK-69-108 and in the galactic law.

This is partly due to the broad $1,920 \text{ \AA}$ feature ($\Delta\lambda \sim 100 \text{ \AA}$) of stellar origin, being much stronger in the spectra of five of the seven reddened stars than in the comparison stars. This $1,920 \text{ \AA}$ feature is due to a blend of Fe III lines, first detected in the S2/68 spectra of luminous stars and found to be independent of spectral type¹⁹. The structure near $\lambda^{-1} = 5.2 \mu\text{m}^{-1}$ in the mean extinction curve is due to this stellar feature. But when corrected by using interpolated continua for the $1,920 \text{ \AA}$ feature, the extinction near $1,920 \text{ \AA}$ decreased by only a few tenths of a magnitude. Therefore the difference between the mean extinction curve for the seven stars and the galactic law as well as SK-69-108 seems to be real shortward of $2,200 \text{ \AA}$. A possible explanation for the increasing extinction in the far UV for the LMC region around 30 Doradus is that the relative abundance of small (a few hundred ångströms sized) dielectric particles is significantly greater than that in the interstellar medium in the solar neighbourhood.

The difference between the extinction curves for SK-69-108, located in the bar, and the other seven stars may indicate a regional difference in the extinction law in the LMC. Clearly further observations of reddened stars in the LMC bar are needed.

Conclusion

From the study of seven reddened stars located within 2° of 30 Doradus in a region to the north-east of the bar of the LMC, and five comparison stars, the mean extinction curve for this region shortward of $1,600 \text{ \AA}$, shows significantly greater extinction than the mean galactic extinction law. This may be due to a relatively higher abundance of small (few hundred ångströms sized) dielectric particles. However, an eighth reddened star, SK-69-108, shows an UV extinction curve similar to the mean galactic law: but this star is located in the bar of the LMC, suggesting a possible regional variation of the far UV extinction law throughout the LMC.

The interstellar extinction curves of all the stars in the visible and near UV wavelength ranges show no significant differences from the mean galactic extinction law.

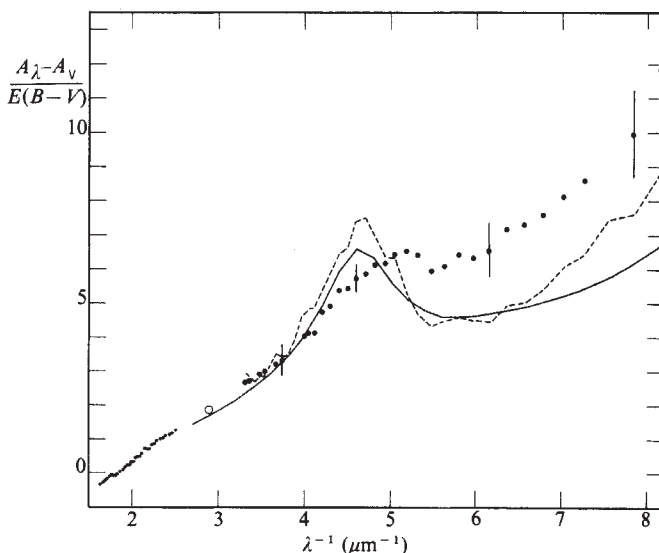


Fig. 3 The mean extinction curve derived from IDS data (dotted line); the mean extinction in the U-band ($\circ$); and the mean UV extinction curve for seven stars situated in a region within 2° of 30 Doradus ($\bullet$). The dashed lines denote the extinction curve for SK-69-108 (situated in the bar). The solid line is the mean interstellar extinction law for the galaxy.

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An extensional origin for the Buchan and Witchground Graben in the North Sea

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Seismic refraction and subsidence observations are used to explain the heatflow and subsidence properties of the North Sea.

THE theory of plate tectonics has been very successful in describing the creation of oceanic crust and accounting for the heat flow and subsidence anomalies observed over mid-ocean ridges¹. However, the theory has not been able to explain the heat flow or subsidence of continental shelves or of epicontinental basins, in particular the Mesozoic and Cenozoic subsidence of the North Sea², which is considered here.

The main structural elements of the North Sea are the Viking and Central Graben, which underlie the axis of Tertiary sinking of the North Sea basin. These two major graben meet the Moray Firth Basin in the region of the Forties oil field, which also marks the confluence of the Buchan and Witchground Graben. The North Sea has been a centre of subsidence since the Devonian and although the graben system was initiated in Permo-Triassic times, most of the extension occurred from the Jurassic to Mid-Cretaceous³. Major rifting seems to have died out by the start of the Upper Cretaceous, giving way to a broad, general subsidence which allowed the accumulation of chalk, sands from the north-west and finally a thick sequence of Tertiary clays, shales and siltstones.

Although there are many models to account for the formation of shelves and inland basins (see refs 4–6), the most attractive are those based on the concepts used in describing the evolution of lithosphere as it moves away from the mid-ocean ridges^{7–9}. In particular, on the basis of a high heat flow and a shallow Moho in the Aegean Sea, McKenzie^{8,10} has suggested a simple stretching mechanism to account for the formation of sedimentary basins. In this model, thinning of the lithosphere occurs as a result of large scale extension whereby the surface area is increased by a factor b . If the extension is rapid and lithosphere is conserved, both the crust and the lithosphere decrease in thickness by a factor of $1/b$, causing a fault controlled isostatic subsidence of the surface and creating a thermal anomaly by the passive upwelling of hot asthenospheric material. As the thermal anomaly decays by conduction through the crust, the lithosphere thickens and subsides allowing, in the absence of further extension, the formation of a saucer shaped sedimentary basin. It can be shown⁸ that the heat flow and subsidence history of a basin can be predicted as a function of the extension factor. If crustal material is conserved, a measurement of the depth to the Moho will indicate the amount of extension when compared with the

Moho depth beneath a non-extended region. We show here that the decrease in Moho depth and increase in subsidence beneath the Witchground and Buchan Graben can be accounted for by a mechanism involving between 50 and 100% stretching of the lithosphere from the Jurassic through to the Mid-Cretaceous.

Seismic refraction data

The Marine Group at Cambridge University undertook the investigation of the velocity structure of the crust and upper mantle along a line parallel to and west of the main axis of Tertiary sinking in the North Sea (Fig. 1). The refraction experiment involved the shooting of three lines into sea bottom seismometers designed and built at Cambridge specifically for this experiment¹¹. The main line, reversed over 400 km, was fired across the Witchground and Buchan Graben and was complemented by a shorter crustal control line at each end. Crustal and upper mantle phases were identified from the seismograms and interpretation of the travel time information was made using the method of time term analysis (see ref. 12). Using this approach, each shot and receiver location is assigned a delay time, or time term, which is due to the presence of the overburden between the shot or receiver point and the buried refractor. If the velocity structure in the overburden is known, then the delay time to the refractor beneath a particular site may be inverted to a depth value.

The analysis of the Firth of Forth profile revealed three refractors beneath the sediments with velocities of 5.7, 6.2 and 7.2 km s⁻¹. The Moho velocity is ~8.16 km s⁻¹, a value taken from the Main Line as the Moho refraction was not reliably identified as a first arrival on this profile. The overall depth to the Moho is ~30 km compared with 32–33 km observed for the Moho depth beneath the Southern Uplands as revealed by the LISPB experiment¹³. For the crustal control line at the north, a time term analysis of these arrivals, together with the crustal arrivals from Main Line shots, gave good control over the crustal refractors although the quality of the data was not as high as for the Firth of Forth Line. On the East Shetland Platform, the short range refractions gave a phase velocity of ~4.1 km s⁻¹, which is consistent with Devonian Old Red Sandstone at shallow depth. This overlies an upper crustal layer of 5.7 km s⁻¹, which in turn covers a layer of 6.2 km s⁻¹. Both these values are in good

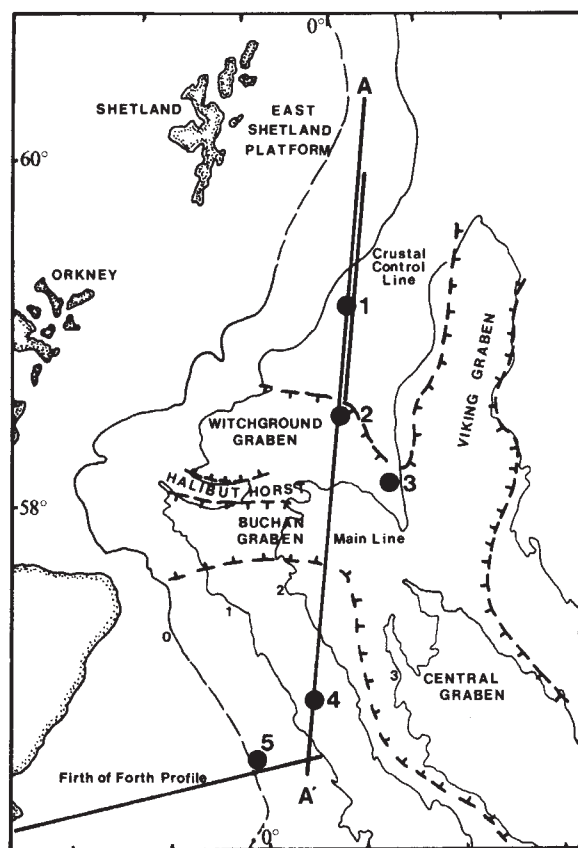


Fig. 1 Seismic refraction lines, drill holes, the major Mesozoic faults and Tertiary isopachs in the Central North Sea. The three refraction lines are the Firth of Forth Line, the Crustal Control Line and the Main Line.

agreement with the velocity structure determined from the Firth of Forth Profile, although difficult correlations make identification of a lower crustal refractor uncertain. On the main north-south line, energetic mantle arrivals were observed on most of the seismograms, but with greatly varying waveform suggesting the superposition of rays. Arrivals were generally well defined and a time term analysis yielded an upper mantle velocity of 8.16 km s^{-1} , which is controlled by good reversed coverage. The delay times to the Moho computed in the analysis increase towards the centre of the line, as does the sediment thickness.

A large number of oil companies have allowed generalised velocity-depth structures to be compiled from their individual well logs. By combining this information with the crustal refraction data already deduced, the Main Line delay times were converted into depths, enabling a seismic section to be constructed (Fig. 2). After stripping off the delay due to the sediments, the Moho has an anticlinal form beneath the sedimentary basin, with the shallowest Moho depth of $\sim 25 \text{ km}$ below the deepest sediments. Further north, the Moho deepens again to $\sim 32 \text{ km}$ beneath the East Shetlands Platform. The anticlinal structure can cause focusing of the refracted rays to produce the varying waveforms observed. The asymmetric position of the Moho anticline with respect to the centre of the profile can also explain the asymmetric amplitude peaks on the Main Line records, if focusing is admitted. The amplitude peaks are at receiver R3 from the northern shots and receiver R8 from the southern shots¹⁴.

Subsidence data

Basement subsidence as a function of time was estimated in a suite of five holes across the Witchground and Buchan Graben from lithologic and biostratigraphic logs provided by the

Shell/Esso, Conoco, Mobil and Amoco oil companies. From these data, the generalised lithologic sections (Fig. 3a) were constructed. To calculate the basement depth at a given time, it is necessary to remove the younger sediments, allow for the effects of compaction on the older sediments, compute the isostatic loading effect of the uncompacted sediments and add the water depth at the time of deposition^{15,16}. We have ignored the effects of eustatic sea level changes. In the decompaction process, the base of the Upper Permian salt or anhydrite was taken as being basement. As the regression at the end of the Aptian-Albian is an almost basinwide reflective marker at the beginning of the phase of general subsidence, this event has been taken as the datum for plotting the depths of subsequent time-stratigraphic units. This unconformity is evidence that the North Sea was generally close to sea level at the beginning of the Upper Cretaceous, a view which is also supported by reports that shallow-water faunal assemblages have been found in the uppermost Lower Cretaceous¹⁷. Hence, plots of depth relative to the Aptian-Albian are graphs of approximately true depth below sea level.

In contrast to Clarke², who suggested that the subsidence rate was increasing during the Tertiary, we find that the subsidence varies almost linearly with time (Fig. 3b). When corrected for the depth of deposition of the sediments, as estimated from faunal assemblages by W. A. Berggren and F. M. Gradstein (personal communications) and by analysis of the sands at hole 2, the exponential tail off of subsidence rate with time becomes more pronounced. Along the profile the subsidence varies from about 500 m to $\sim 1,300 \text{ m}$, with the greatest amount of subsidence in the centre of the graben which coincides with the shallowest Moho depth.

Discussion

An estimate of the stretching factor can be obtained from the seismic section, assuming that stretching stopped at the base of the Upper Cretaceous and that crustal material has been conserved. The depth from base Upper Cretaceous to the Moho at R5 is $\sim 20 \text{ km}$, while under the East Shetlands Platform (or the Southern Uplands) it is roughly 32 km . This indicates a thinning of about 0.6 or a stretching of 1.6. If the value of 35 km for the depth to the Moho beneath the Shetland Islands is used as the estimate of the thickness of the unstretched part of the lithosphere, a value of 1.7–1.8 is obtained for the stretching factor.

Estimates of the amount of stretching can also be obtained from the amount of post-Lower Cretaceous subsidence. The observed subsidence of the five holes was examined and compared with that predicted by the model for stretching factors of 1.25, 1.5 and 2.0. The subsidence on the flanking blocks of the graben system indicates only slight stretching whereas the subsidence curves within the graben suggest stretching values of between 1.5 and 2.0, which covers the range of values given by the seismic evidence.

The stretching mechanism is supported by the heat flow which is higher than normal¹⁸. The observed values are obtained from extrapolated Bottom Hole Temperatures and estimates of thermal conductivities, and could well be too high. Reducing these values by only 10% gives a value that is close to that expected if 35 km of continental crust with a mean radiogenic component of heat flux of $0.8 \mu\text{cal cm}^{-2} \text{ s}^{-1}$ is stretched by a factor of between 1.5 and 2.0 (Table 1).

Another observation accounted for by this mechanism is the 2–3 km of Middle Jurassic to Lower Cretaceous sediment observed within the graben itself. The initial subsidence is probably dominantly fault controlled and occurred during the extensional process. It is caused by isostatic adjustment as a result of the replacement of light crust by denser asthenospheric material. Given the amount of extension deduced from the seismic and subsidence data, the amount of this initial fault controlled subsidence within the graben may be computed from

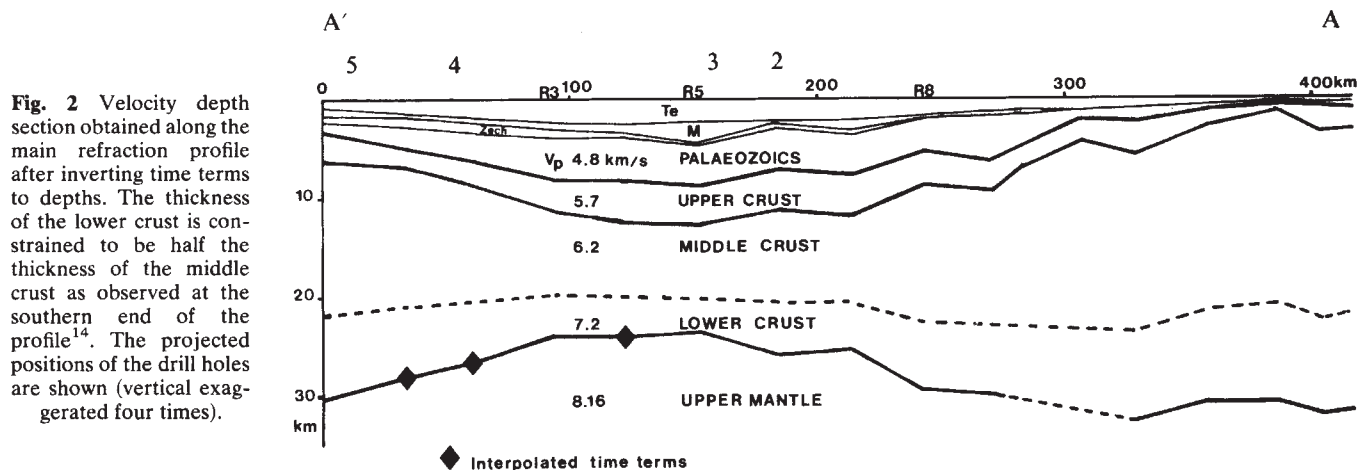


Fig. 2 Velocity depth section obtained along the main refraction profile after inverting time terms to depths. The thickness of the lower crust is constrained to be half the thickness of the middle crust as observed at the southern end of the profile¹⁴. The projected positions of the drill holes are shown (vertical exaggerated four times).

the model^{8,16}. For a stretching factor of 2, ~1.2 km of water-filled or 3 km of sediment-filled subsidence would result. This is very close to the amount of subsidence observed.

Because of the agreement between the observed values of Moho depth, subsidence history and heat flow with the values predicted by the extensional mechanism, we would propose the following model to explain the major features of the development of the North Sea basin. The graben system was emplaced during the Permo-Triassic but with relatively little extension. From the time of the Jurassic through to the Lower Cretaceous, there was significant radial extension with the three parts of the graben system extending by a factor of ~2. The upper crust at

least underwent brittle failure, thinning by a factor of ~2 and producing isostatic subsidence within the graben. When extension terminated, a general subsidence, caused by thermal relaxation through the crust, continued and the present saucer-shaped basin developed (Fig. 4). It is not clear from the observations whether the lithosphere extended as much at depth as it did at the surface. If this were the case, the ductile part of the lithosphere could have thinned by less than the brittle crust but over a greater areal extent. Such deformation would replace cold lithosphere by less dense, hot asthenosphere and would cause the graben flanking blocks to rise during extension. The consequent erosion could explain the absence of Jurassic and

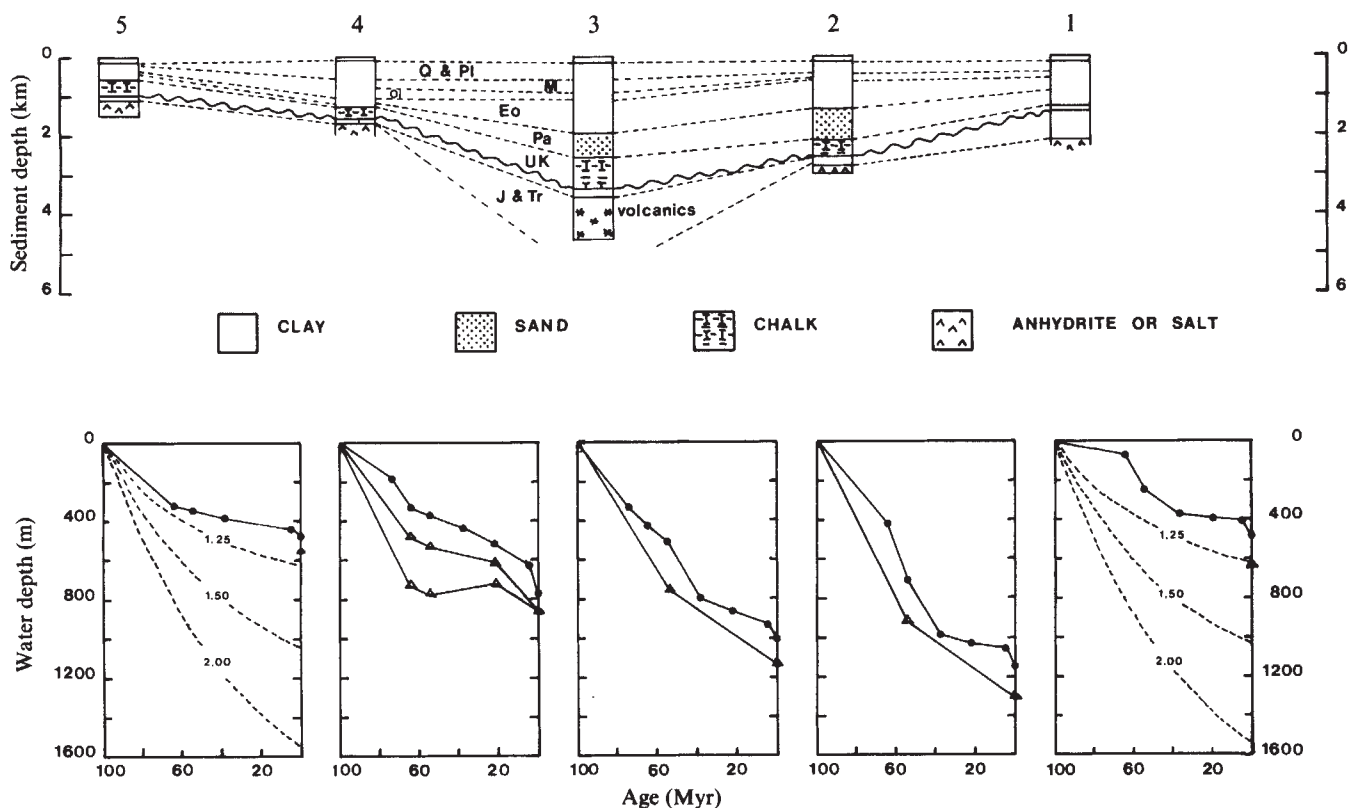


Fig. 3 A lithologic cross-section of the Witchground-Buchan Graben complex showing five wells below line AA' on Fig. 1. The letters on the cross-section represent standard stratigraphic stages and the symbols represent the different lithologies. (Explicitly: J, Jurassic; Tr, Triassic; UC, Upper Cretaceous; Pa, Palaeocene; Eo, Eocene; Ol, Oligocene; M, Miocene; Pl, Pleistocene and Q, Quaternary.) The inferred post-Upper Cretaceous basement subsidence (●), corrected where possible for depth of deposition (△), is shown below each well. The thermal subsidence curves expected after 25% ($b = 1.25$), 50% ($b = 1.50$) and 100% ($b = 2.00$) instantaneous stretching at 100 Myr are shown on the outer two plots of basement subsidence.

Lower Cretaceous sediments on these blocks. However, the presently available seismic, eustatic and subsidence data are too imprecise to allow the inference that such sub-crustal flow has actually taken place.

This model has been proposed to account for the development of the North Sea basin in the absence of a full discussion of alternative mechanisms. We are drawn to this mechanism in preference to models invoking thermally or gravitationally induced metamorphism in the crust or upper mantle because of the observed decrease in the rate of subsidence with time. Purely flexural subsidence in response to gravitational loading predicts a deeper Moho beneath the thickest sediments, which is inconsistent with the seismic evidence. Crustal attenuation by supra-crustal erosion is rejected because of the presence of thick sequences of pre-Upper Cretaceous sediments over much of the present North Sea basin. The effects of some or all of these mechanisms may be observed in the geological record of the North Sea but we believe that the dominant mechanism in the formation of the basin is that of lithospheric attenuation due to extension.

A major responsibility of the model is to account for the required extension in the geology of the North Sea. This cannot be done by back-tracking such high angle faults as are inferred from most multi-channel seismic lines. However, hitherto

Table 1 Comparison of observations from the centre of the graben with values predicted by the stretching model, for two stretching factors

	Observed	Model prediction	
		1.5	2.0
Fault controlled subsidence, Jurassic to Lower Cretaceous	200–800 m	800 m	1,200 m
Corrected thermal subsidence, Upper Cretaceous to Present	1,100–1,300 m	1,000 m	1,500 m
Type of thermal subsidence	Exponential	Exponential	
Moho depth below Upper Cretaceous	20 km	23 km*	17.5 km*
Heat flow ($\mu\text{cal cm}^{-2} \text{s}^{-1}$)	1.70 ± 0.35	$1.47 \pm 0.3^\dagger$	$1.41 \pm 0.3^\dagger$

* Assuming 35 km thickness of normal crust.

† Assuming $0.8 \mu\text{cal cm}^{-2} \text{s}^{-1}$ radiogenic contribution.

unexpected low angle faults have been observed, and secondary high angle faulting is known to have occurred in the area of the Piper oilfield¹⁹, which may be the last of a series of listric faults such as are proposed by Profett²⁰ to explain the extension in the Basin and Range province of the US. While this difficulty needs to be resolved, we feel that the good agreement between the refraction, heat flow and subsidence data warrants the consideration and further investigation of the model.

Conclusions

The approach we have taken has been simplistic, and we have ignored such complications as flexure of the lithosphere due to sediment loading and eustatic sea level changes. Even though these factors have been neglected, we believe that the method is justified by the results which show that stretching by a factor of between 1.5 and 2.0 in the Jurassic and Lower Cretaceous can account for the shallow Moho, subsidence history and present heat flow across the Witchground–Buchen Graben complex. We feel that this study demonstrates the power of comparing seismic refraction and subsidence observations in unravelling the geologic history of the North Sea basin and we suggest that such comparisons may be as useful in investigating other basins. In particular, these studies may be of value to the oil industry because, if the stretching factor can be determined, it should be possible to predict the palaeo-heat flow and hence the degree of thermal maturation of the individual sedimentary horizons¹⁶.

We thank representatives from the Shell, Conoco, Mobil and Amoco oil companies, for access to well logs which, with their experience and that of P. Ziegler, have led us through the complexities of the Mesozoic and Cenozoic sedimentary history of the North Sea.

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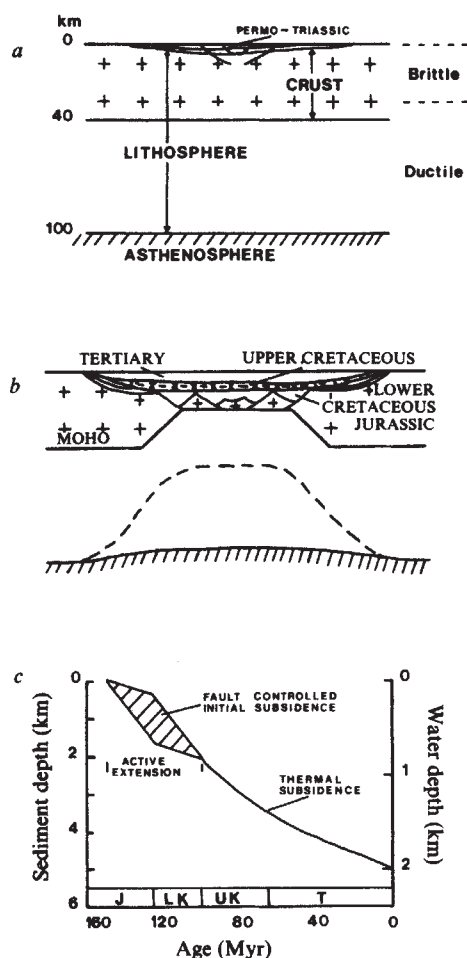


Fig. 4 An outline of the post-Triassic development of the Witchground–Buchen Graben complex. *a*, Initial cross-section before Mid-Jurassic and Lower Cretaceous extension. *b*, Present section after stretching by 100% in the graben. Note the shallow Moho, the localised Jurassic and early Cretaceous section and the widespread Upper Cretaceous and Tertiary. At the beginning of the Upper Cretaceous, the asthenosphere (dashed line) had thinned by a factor of 2. *c*, Conceptual post-Mid-Jurassic subsidence of the centre of the graben complex were the sedimentary supply to match exactly the basement subsidence.

An immunoglobulin heavy-chain gene is formed by at least two recombinational events

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The events of B-cell differentiation can be reconstructed in part through an analysis of the organisation of heavy-chain gene segments in differentiated B cells. A mouse immunoglobulin α heavy-chain gene is composed of at least three noncontiguous germ-line DNA segments—a V_H gene segment, a J_H gene segment associated with the C_μ gene segment, and the C_α gene segment. These gene segments are joined together by two distinct types of DNA rearrangements—a V-J joining and a C_H switch.

THE antibody genes provide a unique opportunity for studying the molecular basis of one pathway of eukaryotic differentiation because the rearrangement of gene segments is correlated with the expression of antibody molecules. Antibody molecules are encoded by three unlinked gene families— λ , κ and heavy chain (H)¹. The λ and κ families encode light (L) chains and the heavy-chain family encodes heavy chains. The light chains are encoded by three gene segments, V_L (variable), J_L (joining) and C_L (constant), which are separated in the genomes of cells undifferentiated with regard to antibody gene expression^{2,3}. During differentiation of the antibody-producing or B cell, the V_L and J_L gene segments are rearranged and joined together while the intervening DNA between the J_L and C_L gene segments remains unmodified^{2,4}. This process of DNA rearrangement is termed V-J joining. During the expression of the rearranged gene in the differentiated B cell, the coding regions as well as the intervening DNA between the J_L and C_L gene segments are transcribed as part of a high molecular weight nuclear transcript. The intervening region is then removed by RNA splicing to produce a light-chain mRNA with contiguous V_L , J_L and C_L coding segments^{5,6}. Recently, we demonstrated that the heavy chain contains three analogous gene segments, V_H , J_H and C_H , which undergo a similar type of V-J joining during B-cell differentiation⁷⁻⁹. Each antibody-producing cell synthesises only one $V_L J_L$ polypeptide sequence and one $V_H J_H$ sequence, which together form the antigen-binding (V) domain of the antibody molecule.

The heavy-chain genes seem to have a special role in the differentiation of the antibody-producing cell as reflected in a second phenomenon known as the switching of heavy-chain constant regions or C_H switching. Antibody molecules can be divided into five different immunoglobulin classes—IgM, IgD, IgG, IgA and IgE—which are determined by one of eight distinct heavy chain genes [that is, C_μ , C_δ , ($C_{\gamma 1}$, $C_{\gamma 2a}$, $C_{\gamma 2b}$, $C_{\gamma 3}$), C_α and C_ϵ]. Each class of immunoglobulin seems to be associated with unique functions such as complement fixation or the release of histamine from mast cells. The process of B-cell differentiation is complex and a variety of conflicting views exists on the transitional stages. It is generally agreed, however, that IgM is the earliest immunoglobulin class that is expressed in the differentiation of a B cell (see ref. 10). The immature B lymphocyte apparently has the capacity to differentiate along a variety of discrete pathways and produce progeny which may switch from IgM expression to the expression of any one of the other classes of immunoglobulins. The terminal stage of B-cell differentiation is the plasma cell, which is committed to synthesise and secrete large quantities of a single molecular species of antibody. Several studies suggest that the specificity or V domain does not change as different immunoglobulin classes

are expressed throughout this differentiation process¹¹⁻¹⁵. During C_H switching, light-chain expression remains unaltered.

We are interested in studying the molecular mechanisms which permit a B cell (or its progeny) to express successive classes of antibody molecules. Here we demonstrate that an α heavy-chain gene derived from a terminally differentiated plasma cell is composed of three noncontiguous germ-line DNA segments. These gene segments are joined together by at least two distinct DNA rearrangements—a V-J joining and a C_H switch.

Organisation of the α heavy-chain gene is altered in differentiated as opposed to undifferentiated DNAs

We have constructed 'libraries' of recombinant phage¹⁶ containing large (12–20 kilobases) inserts of mouse DNA in the vector Charon 4A (ref. 17). These libraries contain sufficient numbers of recombinants ($\sim 10^6$) for there to be a high probability that most single-copy sequences of a given genome are included. We have previously reported the construction of such a library from the DNA of the mouse IgA-producing myeloma tumour M603, and the subsequent isolation and characterisation of clones containing rearranged or differentiated genomic DNA. These clones, CH603 $\alpha 6$ ($\alpha 6$) and CH603 $\alpha 125$ ($\alpha 125$), have V_H and C_α gene segments on a single fragment of DNA^{7,8}. The *EcoRI* restriction maps of these clones are shown in Fig. 1a. The $\alpha 6$ clone has three *EcoRI* fragments—one 7.3 kilobases long containing the V_H gene segment, a second of 5.1 kilobases containing the 5' portion of the C_α gene segment, and a third of 4.4 kilobases containing the 3' portion of the C_α gene segment. Approximately 6.8 kilobases of intervening DNA separate the V_H and C_α gene segments. The $\alpha 125$ clone also has three *EcoRI* fragments—one of 6.5 kilobases which is located on the 5' side of the 7.3- and 5.1-kilobase *EcoRI* fragments also described for $\alpha 6$.

The genomic clones were isolated using a cloned cDNA plasmid representing the entire heavy-chain mRNA of the IgA-producing tumour S107 (denoted S107 cDNA)⁷. The S107 V_H region is about 98% homologous to the M603 V_H region at the nucleotide level⁹. Analysis by the Southern blot procedure^{18,19} of *EcoRI*-digested M603 DNA hybridised to the S107 cDNA probe demonstrates the presence of three *EcoRI* fragments corresponding to those described above in the $\alpha 6$ clone⁷. Thus, the rearranged $\alpha 6$ clone is not an artefact of the cloning or isolation procedures.

When the S107 cDNA probe is used to examine a Southern blot of *EcoRI*-digested sperm or embryo DNA, a somewhat different pattern is observed⁸. In particular, the 5.1-kilobase

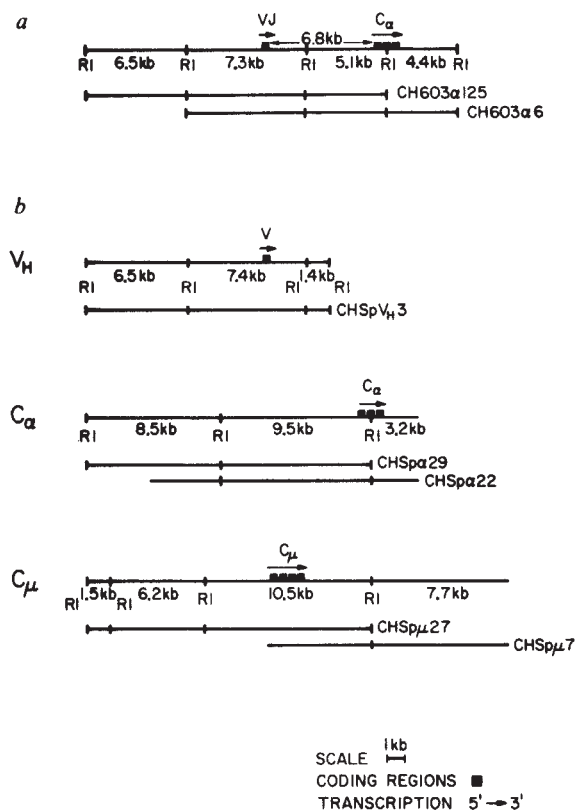


Fig. 1 *a*, α Heavy-chain clones isolated from a genomic library of myeloma M603 DNA. CH603 α 6 and CH603 α 125 are two overlapping clones derived from a partial *Eco*RI library of M603 DNA, constructed using the phage Charon 4A (ref. 7). The position of the respective gene segments, as well as their direction of transcription, was determined by R-loop mapping and restriction analysis^{7,8}. *b*, Germ-line V_H , C_μ and C_α clones isolated from a genomic library of sperm DNA from inbred BALB/c mice. Sperm DNA^{33,34} was partially digested with restriction enzymes in two ways: (1) with a mixture of *Hae*III plus *Alu*I and (2) with *Eco*RI alone. After digestion in conditions designed to maximise the yield of 12–20-kilobase fragments, these fragments were selected on sucrose gradients. The *Hae*III/*Alu*I fragments were methylated with *Eco*RI methylase and blunt end ligated with synthetic *Eco*RI cleavage sites and then cleaved with *Eco*RI essentially as described previously¹⁶ except that *Eco*RI linker ligations were done at 18 °C to lessen the endonuclease degradation. Both types of fragments were then ligated to the isolated arms of the bacteriophage Charon 4A (ref. 16) at 4 °C. The enzymatically recombinant DNAs were packaged *in vitro* using the strains of Sternberg³⁵ and the protocol of Hohn³⁶. The efficiency of packaging was 400,000 plaque forming units (PFU) per μ g inserted DNA in the case of *Eco*RI partially digested DNA and 200,000 PFU per μ g for *Hae*III/*Alu*I digestions. The background of non-recombinant Charon 4A was 25% and <5%, respectively. Approximately 500,000 *Eco*RI and 1,200,000 *Hae*III/*Alu*I clones were constructed and amplified. A library of ~500,000 clones provides a 90% chance of finding a given single copy sequence and a library of 1,200,000 clones a 99% chance³⁷. The use of enzymes that recognise three different sequences reduces the possibility that a particular region of interest is lost from the library because it had too many or too few restriction sites to fall within the sucrose gradient size cut. Libraries were screened^{7,16} with the cDNA clone (see text) S107 or M104E. C_μ cloned cDNA probes identified by DNA sequence analysis^{9,38}. The C_μ clone used extends from codon 300 to the 3'-untranslated region (~1,000 base pairs).³⁸ Independent overlapping clones were obtained from the C_α and C_μ gene segments. Location of the coding regions and the direction of transcription were determined by DNA sequence analyses for the CHSp PC-3 clone, by heteroduplex analyses with the α 6 clone for the CHSp α 29 clone, and by R-loop mapping and restriction analyses of the CHSp μ 27 clone³⁸. The germ-line genomic clones will be denoted μ 27 (CHSp μ 27), V_H 3 (CHSp V_H 3), and α 29 (CHSp α 29).

*Eco*RI fragment containing most of the large intervening sequence and the 5' portion of the C_α gene segment is not present. This observation indicates that the α 6 clone is the product of one or more DNA rearrangements which presumably occurred during B-cell differentiation⁸.

The α gene is composed of at least three different germ-line segments of DNA

In view of the possibility that lymphocytes earlier in the M603 lineage might first have produced IgM molecules and later IgA molecules, we decided to investigate the possible contribution of germ-line C_μ sequences as well as V_H and C_α sequences to the myeloma α gene (Fig. 1a). We constructed several libraries of germ-line DNA (sperm) and proceeded to isolate clones containing V_H , C_μ and C_α gene segments using cloned cDNA probes (denoted S107 V_H , C_α and C_μ) for the corresponding coding regions and the screening procedure of Benton and Davis²⁰. *Eco*RI restriction maps of several germ-line clones are shown in Fig. 1b. We chose sperm (germ-line) DNA for our undifferentiated genomic libraries to eliminate any possibility of DNA rearrangements which may occur in somatic tissues during embryogenesis. The C_α and V_H clones seem to be representative of germ-line sequence organisation because the *Eco*RI fragments containing the corresponding coding regions (in the clones) are identical in size to those found in the Southern blot analysis of undifferentiated DNA with C_α and S107 V_H cloned cDNA probes—9.5 and 7.4 kilobases, respectively (Fig. 2a–d). The V_H and C_α probes were derived from restriction fragments of the S107 cloned cDNA⁷ extending from approximately the 5'-untranslated region to codon 108 (V_H) and from codons 108 to 274 (C_α). Figure 2a shows that a Southern blot of a germ-line

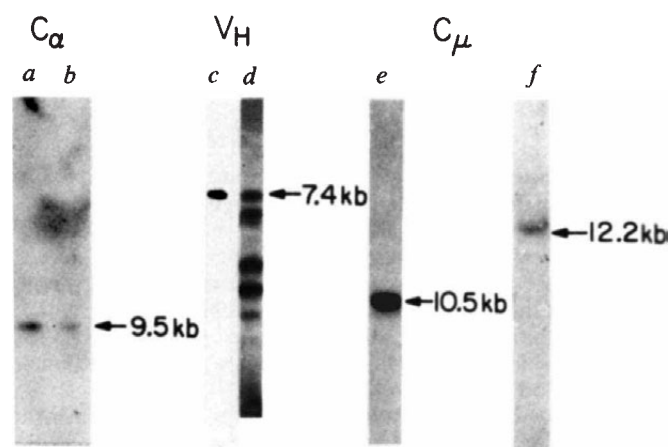


Fig. 2 Southern blot analyses of C_α , C_μ and S107 V_H coding regions in germ-line DNA. Approximately 3–20 μ g of sperm DNA or 13-day embryo was digested with *Eco*RI and electrophoresed on a 0.7% neutral agarose gel for 10–12 h at 30–40 V. Gels were then blotted according to the procedures of Southern and Flavell^{18,19} and hybridised with ³²P nick-translated cDNA³⁹. Washing was for 1.5 h in 1 M NaCl, 1 M Tris pH 8, 0.1% SDS and 0.1% sodium pyrophosphate at 65 °C and for 1 h in 1×SSC, 0.1% SDS and 0.1% sodium pyrophosphate. Lanes *a* and *b* are α 29 and embryo DNAs, respectively, hybridised to the C_α cDNA probe. Lane *c* is V_H 3 DNA hybridised to the S107 cDNA probe. Lane *d* is sperm DNA hybridised to the S107 V_H cDNA probe. This probe cross-reacts with eight or nine closely related V_H gene segments⁸. Lanes *e* and *f* are μ 27 and sperm DNAs, respectively, hybridised to the C_μ cDNA probe. Identical results in all cases were obtained with BALB/c 13-day embryo DNA or sperm DNA as has been found for a V_α gene³⁴. Sizes (given in kilobases) were determined by comparison with restriction fragments of PBR322 (ref. 40) or by the use of PBR322 multimers generated by *Bam*HI cleavage of PBR322 and limited ligation of the resulting monomer.

C_α clone ($\alpha 29$) digested with *Eco*RI and hybridised with the C_α cDNA probe yields a 9.5-kilobase fragment. A similar analysis of embryo DNA produces a band of identical size (Fig. 2b). Southern blots of a germ-line V_H clone (V_{H3}) and embryo DNA digested with *Eco*RI and hybridised to a S107 V_H cDNA probe gave, respectively, a 7.4-kilobase fragment (Fig. 2c) and several fragments including a 7.4-kilobase fragment (Fig. 2d). An important question is the relationship between the germ-line V_{H3} and myeloma M603 V_H coding regions because there are at least eight V_H gene segments that hybridise to the S107 probe⁸. The germ-line V_{H3} DNA sequence codes for the S107 V_H protein sequence⁹ and, accordingly, differs from the M603 V_H region by a minimum of four base changes leading to three amino acid substitutions²¹. Thus, the germ-line V_{H3} and the myeloma M603 V_H gene segments may be encoded by two distinct germ-line V_H gene segments or the V_{H3} gene segment may give rise to the M603 V_H gene segment by somatic mutation and selection. As we shall show subsequently, the V_{H3} clone seems to be indistinguishable from the M603 clone in the V_H coding region and in more than 11-kilobases of 5'-flanking sequence by heteroduplex and restriction enzyme analyses. Therefore, our analyses of the localisation of V_H sequences in the myeloma $\alpha 6$ clone are valid because the germ-line V_H clone serves as a probe for the M603 V_H gene and its attendant 5'-flanking sequence.

The *Eco*RI fragment of the germ-line C_μ clone containing the C_μ coding region is smaller (10.5 kilobases; see Fig. 2e) than its

counterpart seen on a Southern blot analysis of sperm DNA with a cloned μ cDNA probe (12.2 kilobases; see Fig. 2f). We believe this discrepancy arises from one (or more) deletion(s) in the DNA flanking the C_μ coding region during the propagation of the recombinant phage. In isolating μ clones from the M603 library, we obtained several 9.5–10-kilobase *Eco*RI fragments containing C_μ coding regions, whereas Southern blot analysis of M603 DNA with a cloned C_μ cDNA probe demonstrated a genomic fragment of 12.2 kilobases, as in the sperm DNA (data not shown). Attempts to isolate C_μ clones from a library of mouse liver DNA have led to similar results (N. Newell and F. Blattner, personal communication). We will present restriction enzyme data below which demonstrate that this apparent deletion in the μ clone does not affect our general conclusions.

The germ-line V_H , C_α and C_μ clones were compared with the myeloma $\alpha 6$ and $\alpha 125$ clones by heteroduplex analysis. Representative heteroduplexes from each of these comparisons show extensive homologies. The germ-line V_H clone shares approximately 11.6 kilobases of homology with the myeloma $\alpha 125$ clone (Fig. 3a). This homology extends from the 5' end of the $\alpha 125$ clone up to and including the V_H coding region. The germ-line C_μ clone shows 5.0 kilobases of homology with the large intervening sequence of the myeloma $\alpha 6$ clone (Fig. 3b). Starting at its 3' end, the germ-line C_α clone has about 6.4 kilobases of homology with the myeloma $\alpha 6$ clone (Fig. 3c). The heteroduplex measurements for these analyses are given in Table 1.

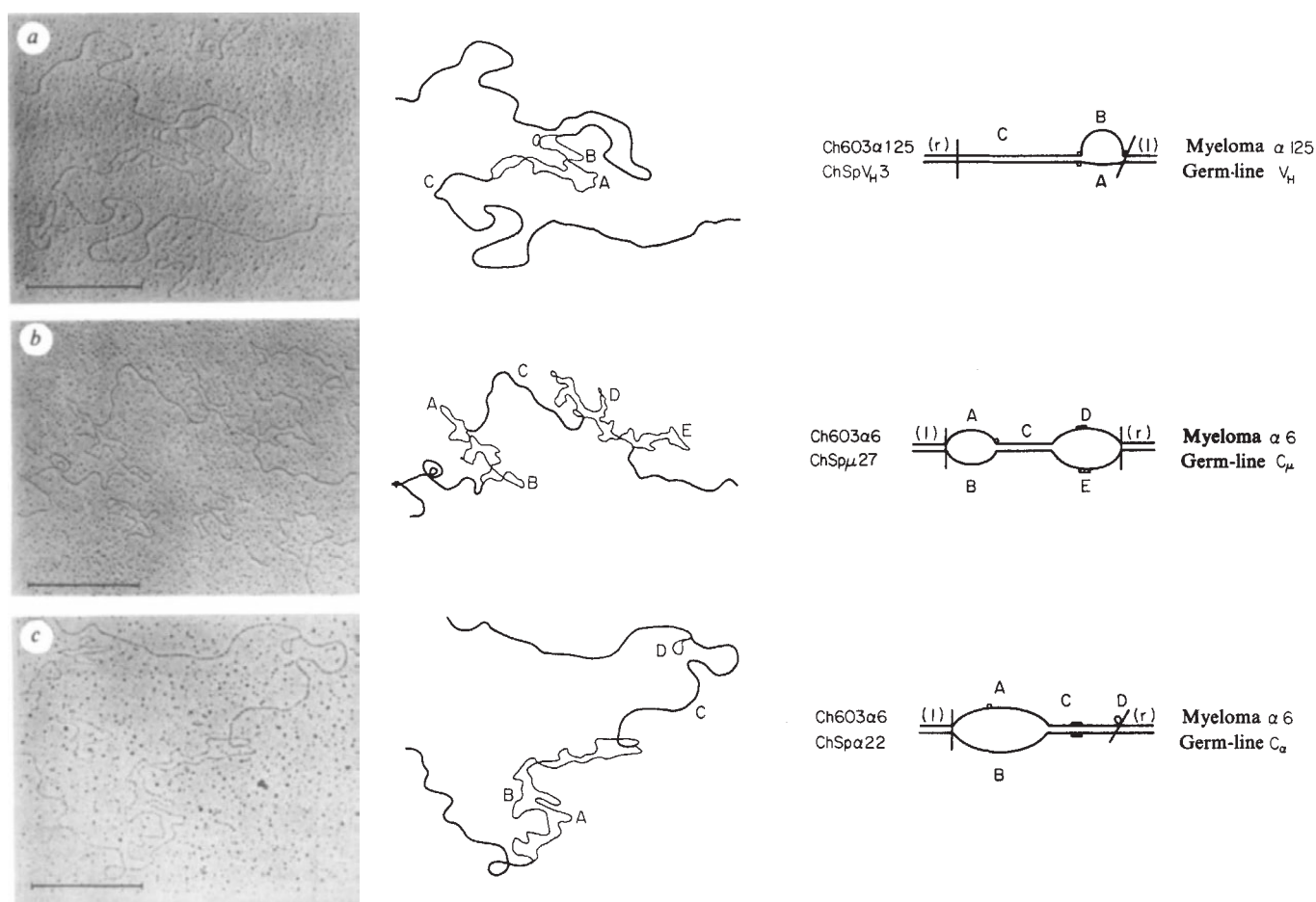


Fig. 3 Heteroduplex analyses of germ-line and somatic clones. The electron micrographs are shown on the left, tracings of these heteroduplexes in the middle and diagrammatic representations on the far right. *a*, Myeloma $\alpha 125$ /germ-line V_H PC3. *b*, Myeloma $\alpha 6$ /germ-line $\mu 27$. *c*, Myeloma $\alpha 6$ /germ-line $\alpha 22$. Letters A–E indicate single-strand and double-strand regions for which measurements are given in Table 1. In corresponding line drawings, (r) and (l) refer to the right and left arms of the λ vector. Blocks indicate coding regions in these figures. CsCl purified phage particles were treated with 0.1 M NaOH for 10 min at 20 °C to lyse the phage and denature the DNA simultaneously. After neutralisation of the mixture, the DNA was allowed to reanneal in 50% (v/v) three times recrystallised formamide for 45 min at 20 °C before spreading for electron microscopy⁴¹.

Table 1 Measurements of heteroduplex molecules

Heteroduplex	No. of Molecules	Distance in kilobases				
		A	B	C ⁺	D	E
<i>a</i> Myeloma $\alpha 125$ / germ-line V_{H3}	35	4.5 $\pm$ 0.4	7.2 $\pm$ 0.7	11.6 $\pm$ 0.4		
<i>b</i> Myeloma $\alpha 6$ / germ-line $\mu 27$	30	4.8 $\pm$ 0.5	5.1 $\pm$ 0.5	5.0 $\pm$ 0.4	6.7 $\pm$ 0.4	7.5 $\pm$ 0.5
<i>c</i> Myeloma $\alpha 6$ / germ-line $\alpha 22$	26	9.3 $\pm$ 0.5	10.4 $\pm$ 0.9	6.4 $\pm$ 0.5	1.2 $\pm$ 0.2	

Measurements were standardised relative to two circular DNA molecules on the same grid [single-strand $\Phi X174$ DNA (5,375 bases) and double-strand pBR322 DNA (4,365 base pairs)]. Letters refer to regions indicated in Fig. 3. The complete clone designations are given in Fig. 3. In heteroduplex *a*, C refers to the region of duplex between the germ-line V_H clone and the myeloma clone. A and B are the non-homologous single strands of these clones. Similarly, C for heteroduplexes *b* and *c* refers to the duplexes formed between myeloma and germ-line C_μ or C_α clones.

Comparative restriction analyses confirm and extend the heteroduplex data discussed above. A detailed restriction map for the M603 myeloma clones was obtained by double digestion with pairs of restriction enzymes and is shown in Fig. 4*a*. To compare the placement of these cleavage sites with those of the germ-line clones, the 5.1-kilobase restriction fragment of the $\alpha 6$ clone (Fig. 1*a*) that spans the region joining the germ-line C_α and C_μ sequences was subcloned. Using this fragment as a probe, detailed restriction comparisons of the myeloma $\alpha 6$ clone and the germ-line C_μ and C_α clones were made. Representative data are shown in Fig. 5 for these comparisons. Figure 5*a*, *b* and *c* represents *HincII* plus *EcoRI* digestions of the myeloma $\alpha 6$ 5.1-kilobase RI fragment, the germ-line $\alpha 29$ clone and the germ-line C_μ clone, respectively. These digests were electrophoresed on an agarose gel, blotted onto a nitrocellulose filter and hybridised with the labelled 5.1-kilobase RI fragment. This enables homologous restriction fragments to be identified rapidly and precisely (arrows indicate identical fragments). Figure 5*d*, *e* and *f* shows a similar analysis using *HindIII* plus *EcoRI* digestions, respectively, of the myeloma 5.1-kilobase subclone, the germ-line $\alpha 29$ clone and the germ-line $\mu 27$ clone. These data, as well as additional restriction analyses of the germ-line V_{H3} clone, demonstrate that 4 out of 4 restriction sites in the germ-line V_{H3} clone, 10 out of 10 sites in germ-line $\alpha 29$ clone and 9 out of 10 sites in the germ-line $\mu 27$ clone corresponded exactly to those found in the myeloma $\alpha 6$ clone (Fig. 4). Not only do these restriction analyses independently confirm the heteroduplex results, but they also suggest that the component germ-line sequences of the $\alpha 6$ clone are very similar to their germ-line counterparts. Thus, the heteroduplex and restriction analyses demonstrate that the germ-line V_H gene segment and its 5'-flanking sequence, although not identical to its M603 counterpart, are very similar and may be used to analyse V_H gene segment organisation in the myeloma M603 clones.

DNA sequence analyses of the myeloma $\alpha 6$ clone and the germ-line $\mu 27$ clone have demonstrated that the heavy-chain gene family does have distinct V_H and J_H gene segments in the germ line⁹. Moreover, the germ-line J_H gene segment corresponding to that expressed in the myeloma $\alpha 6$ clone is associated with the germ-line C_μ gene⁹. This J_H gene segment contains the *HhaI* site that marks the end of the homology between the germ-line $\mu 27$ clone and the myeloma clones (Fig. 4). Accordingly, the distinct germ-line V_H and germ-line J_H gene segments are rearranged in the myeloma clones in a manner analogous to the V_L and J_L gene segments of myeloma light chain genes^{2,3}.

Although it seems unlikely, the M603 J_H gene segment and flanking sequences could have been fused to a germ-line C_μ clone as the result of some cloning artefact. To eliminate this possibility, we decided to demonstrate the germ-line association of the J_H flanking sequence and C_μ sequences by Southern blot analyses. On different slots of the same agarose gel, we electrophoresed mouse sperm DNA cleaved with either *HincII* or *EcoRI*, transferred these DNAs to a nitrocellulose filter and hybridised one lane of each digest with a C_μ probe and a second lane with the 5.1-kilobase *EcoRI* fragment from the intervening sequence of the myeloma $\alpha 6$ clone (Fig. 6). Figure 6*a* and *b* shows Southern blots of *EcoRI*-digested sperm DNA hybridised with the C_μ cDNA probe and the 5.1-kilobase *EcoRI* subclone from the $\alpha 6$ clone, respectively. In both lanes, a 12.2-kilobase band corresponding to the germ-line C_μ fragment can be identified. In a second digest (*HincII*) of mouse sperm DNA, a similar result is obtained with both the C_μ probe (Fig. 6*c*) and the 5.1-kilobase *EcoRI* subclone (Fig. 6*d*) hybridising to a 9.3-kilobase *HincII* fragment. The 9.5-kilobase *EcoRI* band in Fig. 6*b* and the 5.0-kilobase band in Fig. 6*d* correspond to C_α restriction fragments. In each case, one of the two bands from the 5.1-kilobase *EcoRI* probe co-migrated with the single C_μ DNA fragment. This analysis shows that at least part of the intervening sequence from the myeloma $\alpha 6$ clone is adjacent to the C_μ gene in the germ line. Thus, the apparent deletion in the germ-line $\mu 27$ clone is not a significant factor in our discussion.

A summary of these analyses is presented in Fig. 7. The myeloma $\alpha 6$ clone is composed of three noncontiguous germ-line gene segments: (1) a V_H gene segment and its 5'-flanking

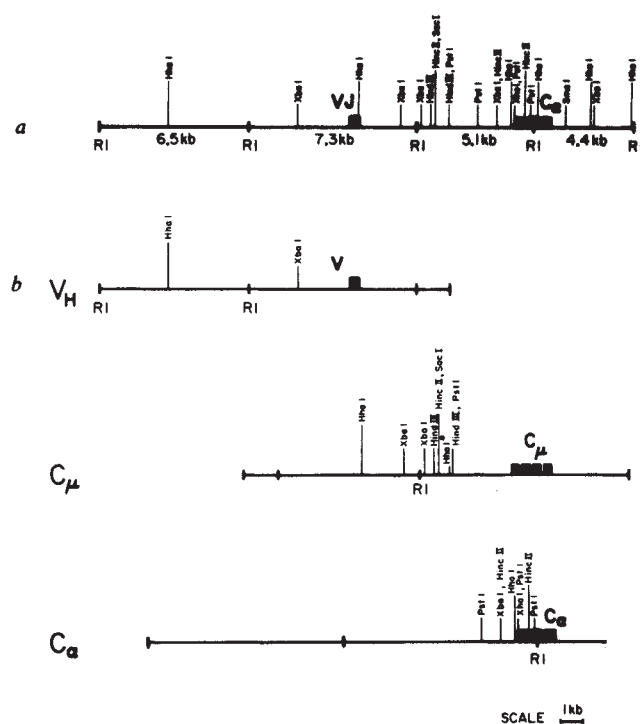


Fig. 4 *a*, Restriction map of myeloma clones $\alpha 6$ and $\alpha 125$. Specific cleavage sites were determined using double enzyme digestion and sizing by gel electrophoresis. Size standards were restriction digests of PBR322 and PBR322 multimers (see Fig. 2 legend). *HindIII*, *HincII*, *PstI* and *SacI* cleavage sites are only shown for the 5.1-kilobase *EcoRI* fragment. *b*, Restriction sites corresponding to those of the myeloma clones detected in the V_H , C_μ and C_α germ-line clones (Fig. 1). Restriction sites in regions corresponding to the 6.5- and 7.3-kilobase RI fragments of $\alpha 6$ and $\alpha 125$ were mapped by double digestion as above. All sites within the 5.1-kilobase RI fragment were compared by co-migration and blotting as described and illustrated in Fig. 5. The *HhaI* site marked by an asterisk in the C_μ clone was the one site found not to conform with those of the myeloma $\alpha 6$ clone.

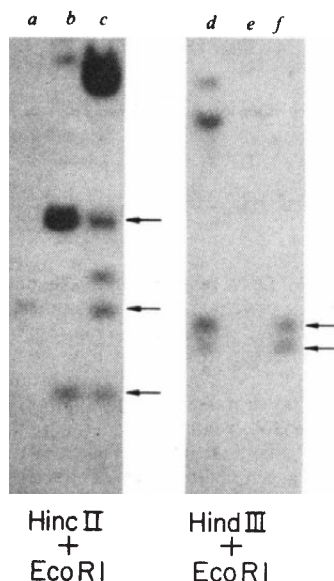


Fig. 5 Comparative restriction digests of the $\alpha 6$ myeloma, germ-line C_μ and C_α clones. Lanes *a–f* show parallel *HincII* + *EcoRI* and *HindIII* + *EcoRI* digests of the 5.1-kilobase *EcoRI* subclone of the $\alpha 6$ clone (Fig. 1*a*) and DNA from the germ-line $\alpha 29$ and $\mu 27$ clones (Fig. 1*b*). Samples were electrophoresed in 1% agarose, transferred to nitrocellulose and hybridised with nick-translated 5.1-kilobase $\alpha 6$ subclone DNA. The co-electrophoresis of restriction fragments allows a large number of different restriction sites to be compared rapidly. In this way, all 16 mapped sites in the 5.1-kilobase $\alpha 6$ subclone were compared with equivalent sites on the $\alpha 29$ and $\mu 27$ clones. Arrows show coincident bands.

sequence, (2) flanking sequences located 5' to a germ-line C_μ gene which includes the J_H gene segment, and (3) the germ-line C_α gene segment with its flanking 3' and 5' sequences. Note that within the limits of the methods used here these three germ-line gene segments and their attendant flanking sequences apparently cover the entire myeloma $\alpha 6$ clone.

The α heavy-chain gene is formed by at least two recombinational events

Two distinct DNA rearrangements have occurred to form the M603 α gene—V-J joining and C_H switching (Fig. 8)⁹. The simplest interpretation of these observations is that the V_H gene segment is first joined to a J_H gene segment linked to the C_μ gene segment. This V-J joining, analogous to that which occurs in light chains, generates a rearranged μ gene that presumably leads to the expression of IgM molecules. V-J joining also commits an individual lymphocyte to the expression of a single V domain that remains invariant throughout subsequent steps of B-cell differentiation. Later, a C_H switch joins the V-J gene segment to the C_α gene segment to create a functional α gene and thus enables the differentiated lymphocyte to express IgA molecules. Thus, the myeloma $\alpha 6$ heavy-chain gene is assembled by two distinct and presumably independent DNA rearrangement events. Our heteroduplex, Southern blot and restriction mapping data show unequivocally that these two rearrangements occur at two distinct sites in the genome. These two sites of rearrangement are termed the V-J joining site and the C_H switch site, respectively.

These data are consistent with a differentiation pathway in which a B cell may switch from IgM to IgA synthesis while expressing the same V domain. We cannot establish that these two DNA rearrangement events occurred at different times, although this supposition is reasonable. We also cannot rule out the possibility of intermediate differentiation states in this B-cell lineage where IgG molecules were produced, although there is no direct evidence for such a stage.

The C_H switch may be explained by any one of several genetic models

Several mechanisms of C_H switching have been proposed^{11,22–26}, many of which are similar to those proposed for V-J joining²⁷. These models can be categorised as involving either DNA rearrangements to replace one constant region with another (successive deletions, excision-insertions or inversions) or the differential processing of a large nuclear RNA transcript containing multiple heavy-chain constant-region genes²⁶.

The RNA processing model seems unlikely as a general mechanism for C_H switching at the level of antibody-secreting plasma cells. High molecular weight nuclear RNAs from three myeloma tumours hybridise only with a cDNA probe complementary to the class of immunoglobulin expressed in that tumour and not with probes from non-expressed immunoglobulin classes²⁸. Moreover, cell fusion experiments hybridising two different myeloma cells demonstrate that the hybrid cells synthesise only parental $V_H C_H$ combinations²⁹, a result in conflict with the simple RNA processing model. Furthermore, neither $C_{\gamma 1}$ nor C_μ DNA probes hybridise on Southern blots with the M603 α clones (data not shown), contrary to one prediction of the RNA processing model.

On the other hand, the evidence presented here strongly supports DNA rearrangement as a fundamental element in the mechanism for heavy-chain switching. As summarised in Fig. 7, a very large segment of C_μ flanking sequence has been brought adjacent to C_α and V_H gene segments in the active α gene of myeloma tumour M603. The creation of the M603 α gene apparently requires two DNA rearrangements (Fig. 8). A C_H switching

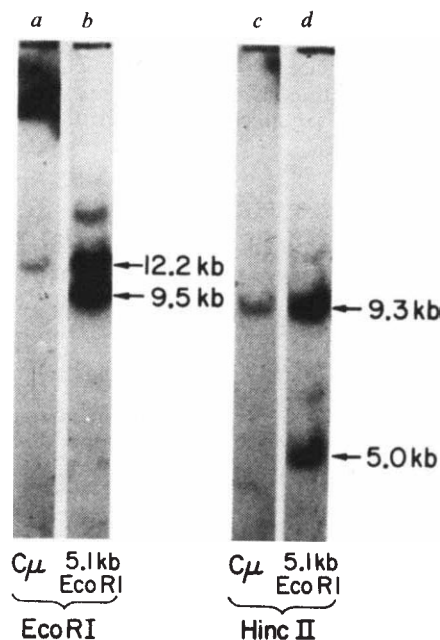


Fig. 6 Southern blots of the 5.1-kilobase *EcoRI* and C_μ fragments. Mouse sperm DNA was digested with *EcoRI* or *HincII* and 3 μ g was loaded onto a 4 mm $\times$ 20 $\times$ 20 cm 0.7% agarose gel, electrophoresed at 40 V for 10 h and blotted as described. Probes used were either the 5.1-kilobase *EcoRI* fragment or a C_μ cDNA clone nick-translated to 4×10^8 c.p.m. per μ g. Washing was as described in Fig. 3 legend except that lanes hybridised with the 5.1-kilobase *EcoRI* fragment were washed further in 10 mM NaCl, 10 mM Tris, 0.1% SDS and 0.1% NaPPi for 2 h at 68 °C to reduce the signal strength of weakly homologous repeats. Filters were exposed for 12 h with an intensifying screen at -80 °C. The faint band above the 12.2-kilobase band in *b* is a partial digestion product. All lanes shown were run on the same gel and blotted simultaneously, alignment being assisted by inclusion of pBR322 multimers as internal standards (see Fig. 2).

DNA rearrangement is not postulated in the RNA processing models²⁶. The data presented here do not distinguish between the various types of DNA rearrangements proposed, but the hybridisation kinetics experiments of Honjo and Kataoka are consistent with a deletional mechanism for the C_H switch²⁵. In addition, recent experiments suggest that V-J joining in mouse λ genes is accomplished by a deletional mechanism⁴. Thus, both types of DNA rearrangement, V-J joining and C_H switching, may arise through deletional mechanisms. If the deletional model is correct for either type of DNA rearrangement, the differentiation of B cells is irreversible because chromosomal information is lost with the excision of each deletional loop of chromosome.

Gene organisation studies may delineate distinct pathways of B-cell differentiation

It will be interesting to determine whether all joined α genes have the same switch site. Southern blot analyses of the DNA from a second IgA-producing myeloma tumour, H8, with the 5.1-kilobase *Eco*RI probe yield a restriction fragment pattern identical to that of M603 DNA (data not shown). In particular, the 5.1-kilobase *Eco*RI fragment (Fig. 1) which contains the switch site seems the same. These data suggest that the expressed α genes in both M603 and H8 myeloma tumours have the same C_H switch site. However, Southern blot analyses of several other closely related IgA-producing tumours do not show a 5.1-kilobase *Eco*RI fragment (M.M.D., unpublished observation). Thus, there may be multiple C_H switch sites for the C_α gene segment. As it seems that B cells producing IgM or IgG may switch to the production of IgA, perhaps distinct C_H switch points reflect distinct pathways of B-cell differentiation. It will also be interesting to determine the location and number of switch sites for other immunoglobulin classes. If each C_H gene segment has a unique site or set of sites for C_H switching, one may be able to trace the distinct pathways of B-cell differentiation by studying the sequence organisation of each functional heavy-chain gene.

DNA rearrangements of antibody gene segments lead to combinatorial amplification of immunoglobulin information

V-J joining and C_H switching are mediated by DNA rearrangements which display combinatorial properties that amplify the germ-line information encoding the antibody gene families. (1) The V and J gene segments of one antibody gene family may be joined in a combinatorial manner to generate diversity in the third hypervariable regions of both κ and heavy chains^{4,8,9,30,31}. For example, mice may have at least 200 V_H and 5 J_H gene segments that may be joined combinatorially to generate 1,000 different V_HJ_H coding regions. (2) One V domain may be combinatorially switched among eight or more different C_H regions to carry out a variety of different effector functions

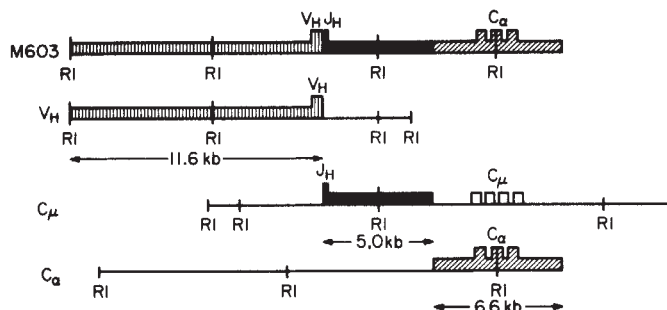


Fig. 7 Origins of the three germ-line components of the myeloma α heavy-chain gene. Various types of shading indicate homology by heteroduplex analyses and by restriction enzyme analyses.

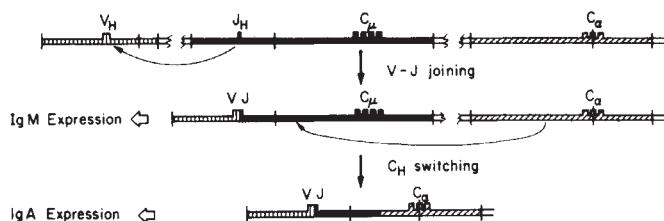


Fig. 8 Two types of DNA rearrangements leading to the creation of the myeloma α heavy-chain gene V-J joining and C_H switching. V-J joining indicates a DNA rearrangement that joins the V_H and J_H gene segments. Because the J_H segments seem to be associated with the germ-line C_μ gene⁹, V-J joining permits a μ chain and IgM molecules to be expressed by the differentiating B cell. C_H switching denotes a DNA rearrangement that replaces the C_μ gene segment with a C_α gene segment. This second rearrangement presumably permits an α chain and IgA molecules to be expressed by the now fully differentiated lymphocyte.

that are directed at eliminating antigen or triggering defensive mechanisms such as complement fixation. Thus, each recognition (V) domain may be switched to many different effector (C) domains. The combinatorial properties of antibody gene segments and polypeptides therefore contribute to several fundamental aspects of the vertebrate immune response—V region diversity and the combinatorial switching of antigen-recognition (V) domains with a variety of different effector (C) domains during B-cell differentiation. Other complex eukaryotic gene families may use similar DNA combinatorial mechanisms for information amplification³².

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The primary structure of 16S rDNA from *Zea mays* chloroplast is homologous to *E. coli* 16S rRNA

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The nucleotide sequence of ribosomal DNA coding for 16S rRNA from Zea mays chloroplast has been determined. A comparison with the 16S rRNA sequence from Escherichia coli reveals strong homology and thereby demonstrates the prokaryotic nature of chloroplast ribosomes from a higher plant.

CHLOROPLASTS of green plants contain 70S ribosomes which differ from the eukaryotic 80S type ribosomes of the cytoplasm¹. The organelle-specific ribosomes mediate translation of organelle-specific mRNAs, which in turn are encoded in chloroplast DNA^{2,3}. Organelle-specific rRNA⁴ and tRNA⁵ species are also encoded in the chloroplast genome, which thus provides the basis for the semiautonomous nature of the organelle. This, along with the various prokaryotic characteristics of the chloroplast-specific translation system, has lent strong support to the endosymbiont hypothesis⁶. This hypothesis postulates that modern chloroplasts are descendants of endosymbiotic prokaryotes—particularly cyanobacteria⁷—which have entered eukaryotic cells during evolution.

Due to a supposedly heavy functional constraint of a highly complex entity such as the ribosome, it seemed likely that its components would have changed comparatively slowly during evolution. Therefore, a comparison of rRNA primary structures from chloroplasts and *E. coli* should provide a quantitative measure of their evolutionary relationship, even if they are separated by a large phylogenetic distance. As an extension of previous work based on T₁-oligonucleotide catalogues^{7–9} of 16S rRNAs, complete sequencing should not only allow the exact positioning of conserved T₁ oligonucleotides, but also a comparison of regions in which larger T₁ oligonucleotides are absent. Furthermore, a differentiation between strongly conserved regions and regions which have gone through changes during the course of evolution will be possible only on the basis of complete sequences. We have, therefore, analysed rDNA from *Zea mays* chloroplasts at the nucleotide level, which should allow deduction of the primary structures of the corresponding rRNA species and comparison with the corresponding rRNA species from *E. coli*. Furthermore, it was anticipated that analysis of sequence homology between organelle and *E. coli* rRNA species would support the prediction of secondary structures if they are common or would help to exclude them if they are not preserved. In this article we report the primary

structure of 16S rDNA from *Zea mays* chloroplast and compare it with *E. coli* 16S rRNA recently analysed by others^{10,11}.

Sequencing of maize chloroplast 16S rDNA

DNA from *Zea mays* chloroplasts is a circular molecule of about 135 kilobase pairs on which two rRNA coding regions are positioned in opposite orientation within two 22 kilobase-pair inverted repeats⁴. Each inverted repeat includes within a 12 kilobase-pair *EcoRI* fragment one copy of a 16S rRNA, 23S rRNA, 4.5S and 5S rRNA gene and a 2 kilobase-pair spacer region between the 16S and 23S rRNA genes. One such 12 kilobase-pair fragment has been linked to the plasmid vector pMB9 within the *E. coli* clone pZmc134 (ref. 4). This clone was therefore used for isolation, mapping and sequencing of the 16S rRNA coding DNA fragments, which are positioned within the left half of fragment *BamHI*-C (Fig. 1)^{4,12,13}.

In Fig. 2 the RNA-like strand of the entire 16S rRNA gene from maize chloroplast is depicted (lower row of each line). Together with maize chloroplast 4.5S and wheat 5S rDNA sequences (T. Dyer *et al.*, unpublished) it represents—to our knowledge—the first sequence analysis of a complete gene originating from a higher plant.

The exact termini of the structural part of the gene cannot be located by DNA sequencing and must await sequencing of the RNA ends. Taking into account the inhomology of the first four positions, it seems not unlikely that the position of the 5'-terminal nucleotide of maize 16S rRNA is different from position 1, although very close to it. At the opposite end positions 1,540 (last homologous position) or 1,541 (non-homologous, but in accordance with the chain end of *E. coli* 16S rRNA) appear as the most likely candidates coding for the 3'-terminal nucleotide of the chloroplast 16S rRNA. Neglecting this uncertainty a chain length of 1,491 results, which, due to several deletions, is 50 residues shorter than the *E. coli* 16S rRNA.

Sequence homology between maize chloroplast and *E. coli* 16S rRNA

When the maize chloroplast 16S rDNA sequence is compared with the 16S rDNA¹⁰ or rRNA¹¹ from *E. coli* (Fig. 2) extensive

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tions 233–240 were predicted to base-pair with positions 122–126. Taking into account that due to the 233–240 deletion one complement of this secondary interaction is completely missing from maize chloroplast 16S rRNA, we consider this possibility unlikely. More likely is a structure in which the base-paired stem of Fig. 3a is connected by a continuous stack to a base-paired region comprising positions 241–294 (see Fig. 6 of ref. 20). A further possible hairpin structure is only reduced in size due to two closely positioned deletions (Fig. 3c).

It will be interesting to see whether similar deletions are present in other chloroplast 16S rRNAs and if the resulting, apparently more primitive structures are accompanied by smaller sizes of other ribosomal components or by even smaller numbers of ribosomal proteins. (The recent observation of an intervening sequence in the *Chlamydomonas* 23S rRNA gene²¹ does not necessarily contradict a more primitive nature of chloroplast rRNA genes, if intervening sequences are interpreted as evolutionary remnants.)

Possible common secondary structures contain compensating base changes

Computer-aided screening of the maize chloroplast 16S rRNA for possible hairpin structures leads to a very large number of hypothetical secondary structures which are difficult to evaluate *per se*. Considering, however, the *E. coli* 16S rRNA species as a not too distantly related mutant species (or vice versa), screening for secondary structures can be narrowed down to structures which are common to both species. Also, common secondary structures gain even higher probability if compensatory base changes are observed in distant positions of the primary structure as exemplified in Fig. 4.

A loop structure near the 5' terminus has been postulated for *E. coli* 16S rRNA²². The homologous structure of maize chloroplast 16S rRNA shown in Fig. 4a contains six base exchanges (as well as one deletion and one insertion, which are considered as neutral) which match each other to allow a loop structure of almost identical size and stability as in the *E. coli* species. We have recently described similar conservation of a 3'-terminal loop structure¹³. In Fig. 4b a double loop structure from the middle portion of 16S rRNA is proposed which also includes a far distance pairing between positions 580–587 and 878–884. This domain would contain altogether 11 exchanges of complete base pairs, that is 22 single base substitutions; although the individual exchanges have occurred at comparatively distant positions of the primary structure, they exactly compensate each other and thereby permit maintenance of a thermodynamically highly stable secondary structure of similar size. Such compensatory base exchanges, therefore, give strong support to the functional significance of the proposed secondary structures, although they would have to be confirmed by more direct experimental evidence. Additional support for the existence of the double hairpin region shown in Fig. 4b comes from kethoxal reaction data²³, which indicate sensitivity of the G residue at position 790, and from T₁ RNase sensitivity data²⁴, which show that the two loop regions are sensitive within the free 30S particle. A reduced sensitivity against kethoxal and T₁ RNase in the presence of coupled 50S particles has led to the prediction^{23,24} that this domain is involved in the interaction of the 30S and 50S subunits at their interphase. In agreement to this, 30S particles modified at position 790 are not found within the 70S particles obtained from subsequent coupling²⁵. From the present data it seems likely that the subunit interaction is mediated by the 768–884 domain of 16S rRNA in the form of

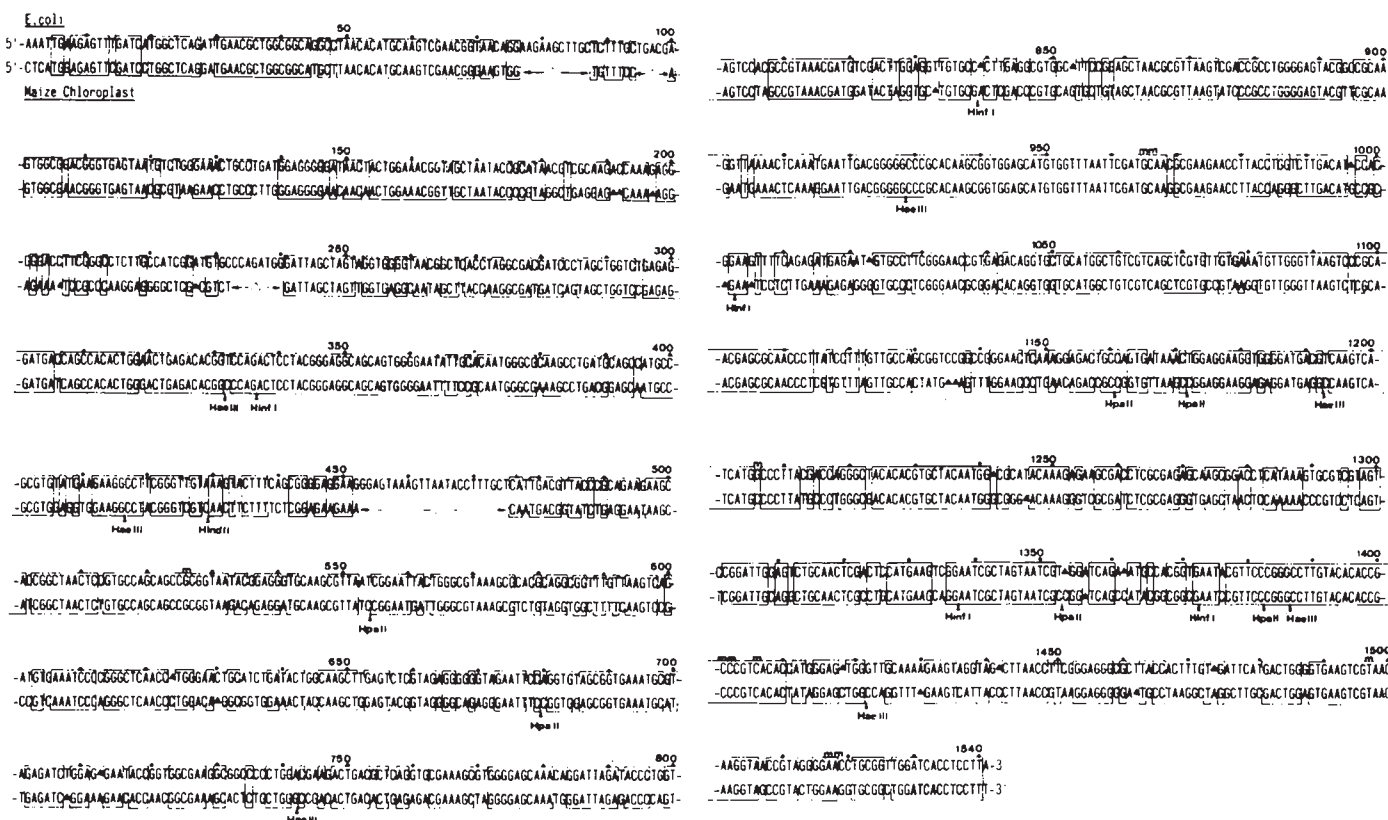


Fig. 2 Nucleotide sequence of 16S rDNA from *Zea mays* chloroplasts. Only the non-coding, RNA-like strand is given in the lower row of each line. The RNA-like strand of *E. coli* 16S rDNA^{10,11} is depicted on top of it for comparison. Homologous sequences are marked by boxes. Triangles within sequences symbolise deleted positions as compared to the other DNA. Numbering refers to *E. coli* 16S rDNA. Due to several deletions not all *E. coli* positions are represented in the maize sequence. The actual numbering of the maize rDNA sequences would therefore be correspondingly lower if this sequence would be counted continuously. It should also be noted that the maize chloroplast rDNA sequence is depicted in opposite orientation as compared to Fig. 1. Cleavage sites for the restriction enzymes used in Fig. 1 are indicated. Methylated positions of *E. coli* 16S rRNA are marked by m.

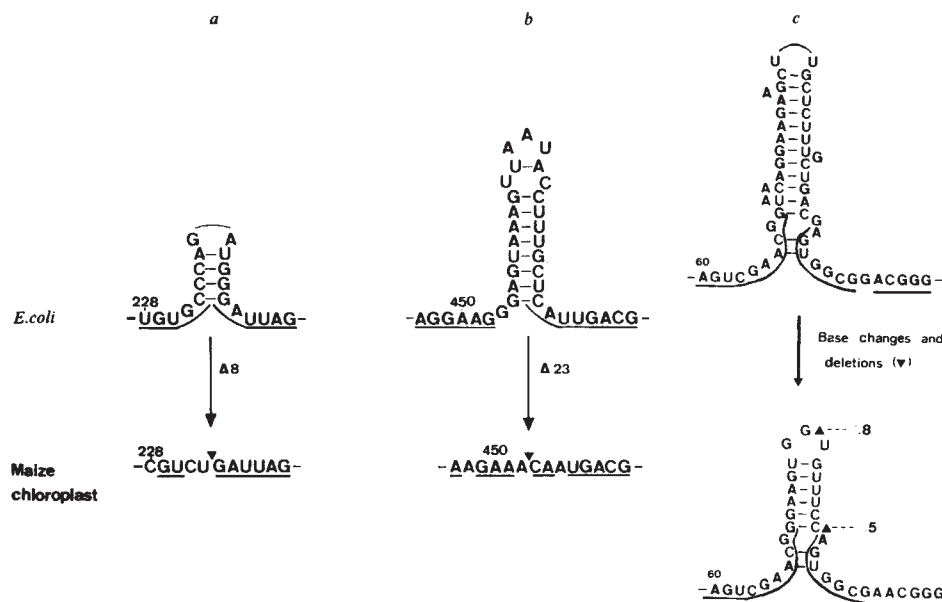


Fig. 3 Postulated hairpin structures deleted (a, b) or reduced (c) in maize chloroplast 16S rRNA as compared to *E. coli* 16S rRNA. Numbers refer to positions in *E. coli* 16S rRNA (see Fig. 2). Homologous positions are marked by underlining. Positions of deletions are indicated by ▼.

the hairpin structures depicted in Fig. 4b. Co-migration of oligonucleotides in a two-dimensional electrophoretic system also supports the existence of secondary structure in the 808–997 region of isolated *E. coli* 16S rRNA²⁰. From these data, however, a secondary structure very different from Fig. 4b was proposed (see Fig. 6 of ref. 20). On the basis of best fit for compensatory base exchanges between *E. coli* and maize chloroplast 16S rRNA we prefer the structure proposed in Fig. 4b.

Compensatory base exchanges also support the proposed secondary structures for the protein S8/S15/16S rRNA interacting site²⁶ and allow a slight refinement of one of the two proposed structures (not shown here, but compare Fig. 7 of ref. 26 with our Fig. 2).

These examples taken together demonstrate that compensating base substitution between functionally related RNA species can provide a valuable criterion for the evaluation of their hypothetically or experimentally deduced secondary structures.

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Note added in proof: A major portion of the yeast 18S rDNA has recently been sequenced²⁹. A comparison of this sequence with 16S rDNA from either *E. coli* or maize shows only very few homologous regions; this indicates a wide evolutionary distance between prokaryotic 16S and eukaryotic 18S rRNA and further substantiates the prokaryotic character of the maize 16S rDNA.

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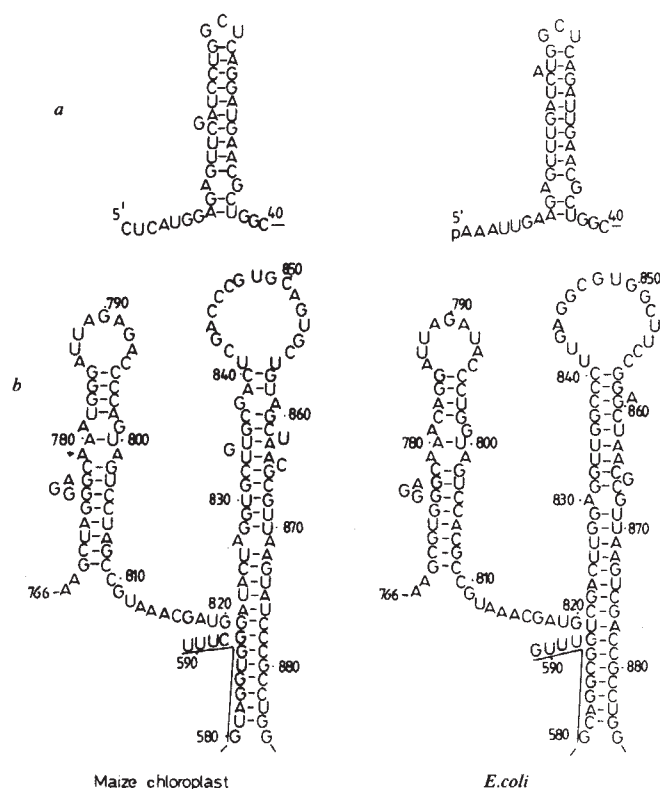


Fig. 4 Postulated secondary structures of 16S rRNA regions which, due to homology and/or compensating substitutions in distant positions of the two chains, show nearly identical dimensions. The two structures to the left represent maize chloroplast rRNA sequences. A possible far distance interaction in b is marked by underlining. Numbers refer to positions of *E. coli* 16S rRNA (see Fig. 2).

Left-handed DNA helices

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The synthetic DNA polymer poly d(GC) · poly d(GC) has been studied by X-ray diffraction of orientated fibres in which it has either the well known (A or B) right-handed conformations or a new left-handed helical conformation. Similar left-handed conformations have been observed with poly d(AC) · poly d(GT) and with poly d(As⁴T) · poly d(As⁴T), demonstrating that the novel structure is accessible to any DNA segment with an alternating purine–pyrimidine base sequence.

SINGLE crystals of d(CpG(pCpG)₂) complexed with spermine and of the high salt form¹ of d(CpGpCpG) contain duplex structures which, if elaborated in a polymer, would lead to a left-handed DNA double helix of pitch ~4.4 nm containing six dinucleotide pairs (A. Rich and R. E. Dickerson, personal communications). Using X-ray diffraction of oriented fibres, we have been studying the structures of sodium salts of poly d(GC) · poly d(GC), a synthetic DNA polymer of high molecular weight (~2 × 10⁶). The polynucleotide is usually found to have one of the common (A or B) right-handed double helical conformations. Occasionally it is found with what must be essentially the same left-handed double helical conformation observed in the single crystal studies: its unit of structure is a dinucleotide, its screw symmetry is 6-fold, and its pitch is 4.35 nm. Moreover, our polymer models which incorporate the same *syn*-G and *anti*-C nucleoside conformations observed by the MIT group give a good account of the fibre diffraction intensities (F. J. Kolpac, A. Rich and G. J. Quigley, personal communications).

The left-handed helices can appear in fibres of poly d(GC) · poly d(GC) in which previously the right-handed B-DNA conformation was observed, and in conditions quite unexceptional with respect to hydration or the type or number of associated cations. Similar structures are observed with polymers containing other alternating purine–pyrimidine base sequences. It would be surprising, therefore, if this novel DNA conformation were not used biologically.

Molecular dimensions and packings

The A form of poly d(GC) · poly d(GC) (Fig. 1a) is not detectably different from that of other DNAs (ref. 2) being an 11₁ helix of pitch 2.82 nm packing in a monoclinic (C2) unit cell with $a = 2.22$ nm, $b = 4.06$ nm, $c = 2.82$ nm, $\beta = 97^\circ$.

The B form (Fig. 1b) has the same symmetry (10₁) and helix pitch (3.35 nm) as in other DNAs (ref. 3). The overall intensity distribution in the diffraction pattern also suggests that the normal B-DNA conformation is unchanged although the orthorhombic unit cell with $a = 3.61$ nm, $b = 3.79$ nm, $c = 3.35$ nm probably contains twice as many DNA chains as the usual cell.

The new form (Fig. 1c) at 44% relative humidity is a 6-fold helix which packs in a hexagonal unit cell with $a = b = 1.91$ nm, $c = 4.35$ nm. The axial translation per (dinucleotide) residue is therefore $h = 0.725$ nm.

The conformation of left-handed DNA

The structures of about two dozen right-handed polynucleotide helices have been examined in detail⁴. All of them are conformationally polymononucleotides. The novel form of poly d(GC) · poly d(GC) is a polydinucleotide conformation-

ally as well as chemically. Using the linked-atom least-squares procedure^{5,6} and assuming that the G and C nucleosides have *syn* and *anti* conformations respectively (as found by the MIT group), we have produced a preliminary model for this structure (Table 1 and Figs 2 and 3) with standard bond lengths and bond angles and a minimum of steric compression. One nucleotide (pC) has the same kind of conformations that are commonly observed in right-handed polynucleotide helices such as A- and B-DNA. The nucleotide pG is quite different not only in the mutual orientation of sugar and base rings but also in the C4'-C5' conformation which is *trans* rather than *gauche*⁺, and the P-O conformations which are *gauche*⁺ rather than *trans* or *gauche*⁻. Nevertheless, this combination of conformation angles yields a structure free of overshort nonbonded contacts.

Morphology

In a polymononucleotide duplex every nucleotide is quasi-symmetrically related to every other one by the operations of the molecular screw axis or the dyad axes in the mean plane of each base pair and midway between each pair. One consequence is that the normals to every base plane make equal angles (γ) to the helix axis. In a polydinucleotide duplex like the novel left-handed DNA this is no longer the case and the guanines ($\gamma_G = 18^\circ$) have quite a different orientation to the cytosines ($\gamma_C = 7^\circ$).

The angle between the guanine and cytosine planes of a base pair (14°) is similar to that found in A-DNA (10°) and B-DNA (13°). The twist axis is tilted 5° from being perpendicular to the helix axis. This tilt is in the same sense as in all members of the B genus of polynucleotide helices⁴. The position of the base pairs relatively close to the helix axis is also like that found in the B genus. Consequently both major and minor grooves are deep. The base pairs are well stacked. As is commonly the case in polynucleotide duplexes most of the overlap involves the central part of the base pairs and not overlap of the heterocyclic rings themselves (Fig. 2). The cytosines are sandwiched relatively effectively between the guanines but these are more exposed.

Another consequence of the molecular asymmetric unit being a dinucleotide is that successive phosphate groups do not have the same radius (R): in CpG $R_P = 0.95$ nm; in GpC $R_P = 0.85$ nm. Nor are the phosphates equally spaced along the polynucleotide chain: in pGp d(P...P) = 0.61 nm, in pCp d(P...P) = 0.64 nm. As a result of this, pairs of proximate phosphates from the antiparallel chains cluster together (Fig. 3). Effective charge neutralisation or shielding is thus important to maintain this structure.

The provenance of the left-handed form of poly d(GC) · poly d(GC)

It is well known^{10,12} that an unusual structure is accessible to poly d(GC) · poly d(GC) and its fragments in aqueous solutions of higher concentration than 2.5 M in Na⁺ or > 0.7 M in Mg²⁺. The predictions of Patel *et al.*¹⁰ with regard to the details of this unusual structure are sufficiently fulfilled by our model for

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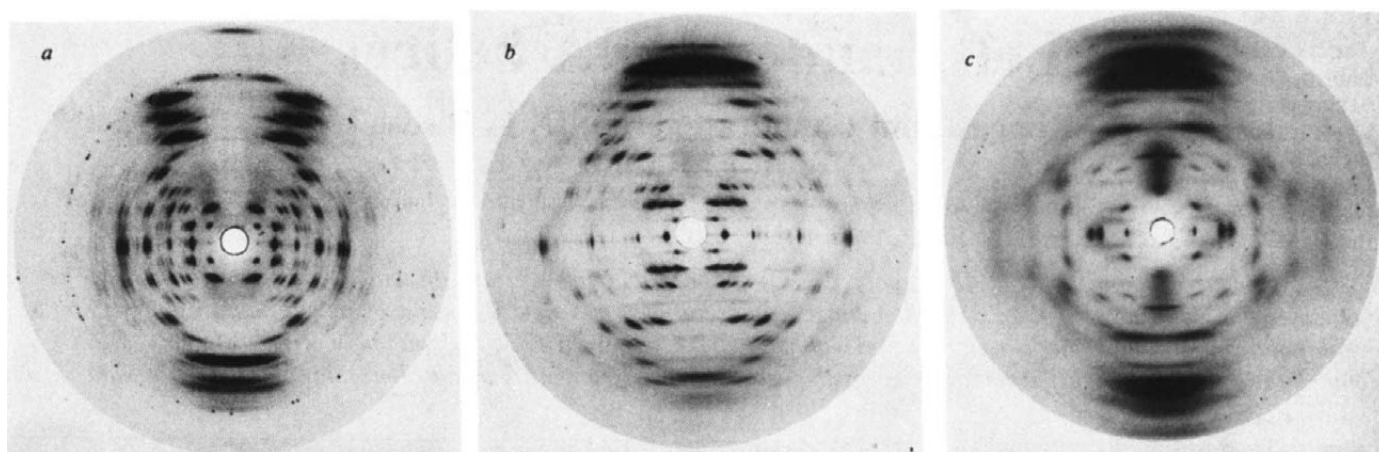


Fig. 1 X-ray diffraction patterns from oriented fibres of poly d(GC) · poly d(GC) in its A form (a), B form (b) and novel form (c). The specimens containing the A and B forms are also polycrystalline so that only Bragg reflections are observed. The symmetry of the new conformation is 226_s and the space group of the diffracting structure probably $P226_s$. Which of the two molecular dyad axes (separated by $c/12$) is made to coincide with a space group dyad appears to be indeterminate. The result is a 'statistical' crystal structure¹³ which gives both Bragg reflections and continuous streaks. The former are predicted to prevail on layer lines 0,2,5,7,9,14 the latter on layer lines 1,6,8,11,13 as observed.

us to be in no doubt that the new structure trapped in our oriented fibres is essentially the one that has been probed spectroscopically in 'high salt' solutions. The circumstances in which we have observed the transition to this new form in fibres supports this conclusion. It appears after prolonged annealing in some fibres prepared with the 3–6% retained NaCl necessary to reproduce the B conformation in typical DNA specimens. The left-handed helix has never appeared in fibres with minimum retained salt. With these, the A-DNA structure is observed initially and persists even after annealing for 30 months.

Apparently, left-handed helical conformations are not confined to poly d(GC) · poly d(GC) but are accessible certainly to other DNAs with alternating purine–pyrimidine nucleotide sequences. We have observed a similar diffraction pattern with one poly d(GT) · poly d(AC) specimen. This implies that pA can, when necessary, mimic the novel pG conformation. This conclusion is reinforced by the structure of poly d(As⁴T) · poly d(As⁴T). These molecules can have 7-fold screw symmetry¹¹ and pack in quasihexagonal unit cells with

$a = b = 1.8\text{--}2.3$ nm and $c = 5.3\text{--}5.4$ nm depending on the relative humidity.

Although Saenger *et al.* interpreted the structure of poly d(As⁴T) · poly d(As⁴T) quite differently, we think that the diffraction patterns are so similar to Fig. 1c that a left-handed structure (Table 1) with $h = 0.76$ nm, $t = -51.4^\circ$ (rather than 0.73 nm and -60°) provides a more plausible solution. Although s⁴T nucleosides have been observed with the *syn* conformation in crystals^{14,15}, our modelling suggests that it is the purine (A) and not the pyrimidine (s⁴T) nucleosides which play the conformationally unorthodox role like G nucleosides in the left-handed helix.

We have tested the possibility that our left-handed structure is accessible to DNAs with other base sequences by exchanging C and G and by imposing the homopolymer sequences of poly d(G) · poly d(C) on our model. Considerable steric compression results in both cases. Left-handed helical secondary structures that would accommodate any nucleotide sequence, therefore, would have to be somewhat different in conformational details.

Table 1 Comparison of conformation angles in the two common right-handed DNA helices with those of two left-handed helices

	A-DNA* (11_1 , $h = 0.26$ nm)	B-DNA† (10_1 , $h = 0.34$ nm)	Poly d(GC) · poly d(GC)‡ (6_s , $h = 0.73$ nm)		Poly d(As ⁴ T) · poly d(As ⁴ T)§ (7_6 , $h = 0.76$ nm)	
			CpG	GpC	s ⁴ TpA	Aps ⁴ T
$\theta(\text{C4}'\text{--C3}'\text{--O3}'\text{--P})$	175°	-133°	-72°	-103°	-92°	-106°
$\theta(\text{C3}'\text{--O3}'\text{--P--O5}')$	-45°	-157°	102°	-91°	99°	-77°
$\theta(\text{O3}'\text{--P--O5}'\text{--C5}')$	-90°	-41°	52°	-110°	60°	-96°
$\theta(\text{P--O5}'\text{--C5}'\text{--C4}')$	-149°	136°	-153°	-168°	-158°	-148°
$\theta(\text{O5}'\text{--C5}'\text{--C4}'\text{--C3}')$	47°	38°	178°	54°	161°	36°
$\theta(\text{C5}'\text{--C4}'\text{--C3}'\text{--O3}')$	83°	139°	76°	147°	80°	135°
$\theta(\text{O4}'\text{--C1}'\text{--N9--C4})$	-154°	-102°	89°	—	85°	—
$\theta(\text{O4}'\text{--C1}'\text{--N1--C2})$	-154°	-102°	—	-159°	—	-154°

Under each helix type are given the molecular screw symmetry and axial rise per asymmetric unit.

* From ref. 7 as adjusted for ref. 8.

† The C2'-endo model of ref. 9 re-refined by S.A. and R.C. (unpublished data).

‡ It is too early to claim that these are the only conformations consistent with the diffraction from poly d(GC) · poly d(GC) but they are similar to those of the core of d(CpG(pCpG)₂) in one of its crystallised duplex forms (G. J. Quigley, personal communication) and to the structure anticipated by Patel *et al.*¹⁰ from their NMR study of d(CpG(pCpG)₃) in solutions of relatively high ionic strength (2.5 M NaCl). Certainly, the diffraction pattern predicted by the model (Figs 2, 3) closely resembles the one observed (Fig. 1c): the crystallographic residual for the Bragg reflections has a value $R = 0.27$.

§ This model for the structure of poly d(As⁴T) · poly d(As⁴T)¹¹ may not be the best solution.

DNA polymorphism

The considerable polymorphism of regular helical DNA duplexes has only recently been established. In the A genus, having polymononucleotides with C3'-endo furanose rings and all conformation angles of the same kind as A-DNA in Table 1, there is little variability in the rotation per nucleotide residue ($30^\circ \leq t \leq 33^\circ$) except for single-stranded molecules when $t = 60^\circ$ is possible¹⁶. However the translation per residue (and therefore the mass per unit length) can vary by as much as 23% ($0.26 \text{ nm} \leq h \leq 0.33$) from A-DNA to poly d(T) · poly d(A) · poly d(T). In the B genus—polymononucleotides with C2'-endo furanose rings and all conformation angles as in B-DNA in Table 1— h is not quite so variable ($0.30 \leq h \leq 0.34 \text{ nm}$) but t varies from its low value of 36° in B-DNA to its high value of 45° in D-DNA¹⁷.

The discovery of a left-handed DNA conformation dramatically extends the range of secondary structures known to be allowed to DNA duplexes and may help to render moot many topological problems thought to be associated with some DNA activities. The known DNA conformations that are substantially overwound or underwound compared with B-DNA could be used to store or shunt locally the rotations needed to wind or

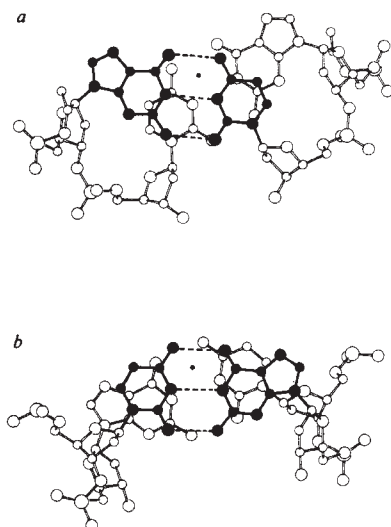


Fig. 2 The base stacking of *a*, GpC and *b*, CpG. The helix axis position is shown by a dot. A molecular dyad axis exists midway between the base pairs in each stack shown. In contrast to the more familiar polymononucleotide structures, the local dyad axis relating the glycosidic links of a base pair does not intersect the helix axis.

unwind a double helix. For example, a segment of left-handed DNA, maintained in a region where the preferred sequences were sufficiently common, could be a store of negative windings. In appropriate conditions these could be used to compensate positive windings and produce a region of melted DNA available for polynucleotide synthesis. Conversely, regions of unwound DNA could condense to give contiguous segments of left-handed and right-handed helical DNA separated by as few as two nucleotides¹⁸. Such a structure might be a manageable substrate for topological isomerisation. Alternatively, the left-handed segment would form a package of negative windings which could be translocated easily even to a distant site.

Overwound and underwound DNA secondary structures could also be associated with the creation or maintenance of supercoiled tertiary structures of either hand. When these are formed or destroyed, compensating changes in secondary structure are required. What these secondary structures might be has now been visualised.

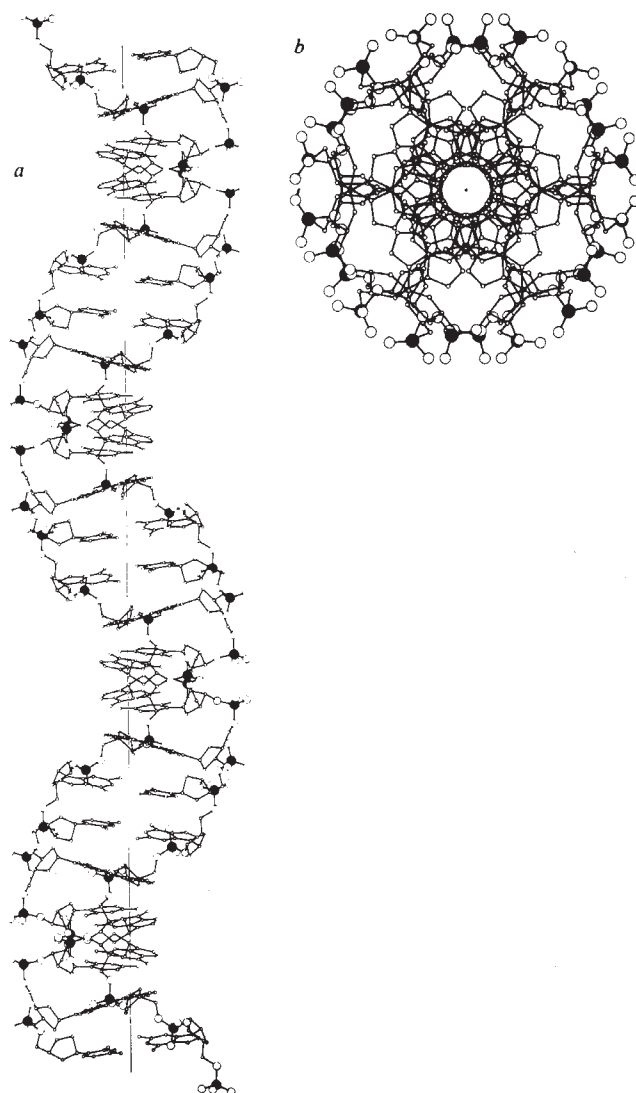


Fig. 3 A two-pitch length fragment of the new form of poly d(GC) · poly d(GC) viewed perpendicular to (*a*) and along (*b*) the helix axis and showing the disposition of the phosphate groups whose atoms are depicted with large circles.

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LETTERS

Is 0406 + 121 the reddest BL Lac object?

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0406 + 121 is a flat-spectrum variable radio source first discovered by Shimmins *et al.*¹ and later studied by Condon *et al.*². While no optical counterpart was identified, similar sources were often found to be either BL Lac-type objects or high redshift QSOs. Recently, Rieke *et al.* detected the object in the near-IR, where it was observed to vary in luminosity over the six-month period between August 1978 and March 1979. A Kitt Peak 4-m plate taken on 29 November 1978 and published by Rieke *et al.* revealed a stellar image ($V = 20.5 \pm 0.4$ mag) at the radio (and IR) position, which lies ~ 30 arc s north of an anonymous spiral galaxy. Rieke *et al.* suggested that 0406 + 121 and some similar sources they detected having steep power law spectra were a possible new type of QSO discriminated against in conventional optical searches. To clarify the nature of 0406 + 121 further, we obtained a spectrum in good seeing conditions on the night of 27 February 1979 using the Steward Observatory 2.25-m telescope and intensified Reticon spectrograph. The object, clearly visible on an intensified TV guider, was wobbled every 80 s between two circular apertures 2.5 arc s in diameter spaced 20 arc s apart. Eighty-five minutes of integration time, calibration with the spectroscopic standard EG 67 (ref. 4) and a HeAr comparison lamp, and eight point smoothing of the 10 Å resolution spectrum gave the results shown in Fig. 1.

The spectrum in Fig. 1 is consistent with a smoothly rising featureless continuum and seems to place 0406 + 121 in the class of BL Lac-type objects. However, the source is unusual in several respects. First, a $\nu^{-\alpha}$ power law fit to the data leads to $\alpha \approx 3.8$. The absorption implied at galactic latitude $b = 28^\circ$ is $A_V \approx 0.11$ mag (ref. 5) so that corrected for reddening, $\alpha \approx 3.7$, to which we attach a conservative error of ± 0.7 . Taken at face value, the optical continuum of 0406 + 121 is thus the reddest known for any object in the BL Lac class. For comparison, α is ≈ 3.2 for AO0235 + 164 and ≈ 3.1 for BL Lac itself, the sources having the steepest previously known spectra⁶.

A second curious property of 0406 + 121 concerns the possible lack of optical variability as compared with that seen in the near-IR. The V flux we derive from Fig. 1 is 20.2 ± 0.3 mag. This agrees closely with the photographic V magnitude measured three months earlier and quoted above. (A further check on this consistency was provided by our own visual estimate off the TV guider!) However, a systematic and substantial decrease in near-IR brightness was observed by Rieke *et al.*: they measured a K ($2 \mu\text{m}$) flux of ≈ 14.0 mag at the end of August 1978; a flux of ≈ 14.3 mag on 11 September 1978; a flux of ≈ 14.9 mag on 10 February 1979; and could not detect the object on 8 March 1979 (nine days after our spectrum was taken) when a 2σ upper limit of ≈ 15.6 mag was obtained.

We have examined all of the published IR photometry of BL Lac sources compiled in Table 1 of Rieke and Lebofsky⁷. In every other instance when luminosity variations are observed, either the optical and near-IR flux change in step, or the optical flux changes over more rapid time scales. In particular, during periods of fading, the optical luminosity has been observed to decrease faster than in the near-IR in AO0235 + 164 (ref. 8) and in B21308 + 326 (ref. 9). If we interpret the Rieke *et al.* $2\text{-}\mu\text{m}$ observations as indicating a gradual and constant decline, then the optical and near-IR continua of 0406 + 121 are decoupled in a manner that is very atypical. This may have important implications in regard to the underlying source energetics (see ref. 10).

Unfortunately, neither of the V magnitudes (which are themselves more uncertain than desirable) were determined simultaneously with the IR data. Thus we cannot rule out the possibility, however unlikely, that our spectrum was taken during an outburst of 0406 + 121 (a common event for BL Lac objects) which then faded rapidly, giving the appearance of constant optical luminosity.

Weak evidence for at least some change in the optical flux of 0406 + 121 over the past 30 years is provided by the absence of the object on either the blue or red Palomar Observatory Sky Survey (POSS); for example, the present brightness above the red POSS plate limit is ≥ 0.5 mag.

In one respect 0406 + 121 does seem similar to its cousins AO0235 + 164 and BL Lac in that the near-IR spectral index seems less steep than in the optical. Physical reasons for such an effect have been considered by O'Dell *et al.*⁸. While again lack of simultaneously obtained data does not permit a conclusive decision, two indirect arguments do suggest the presence of a

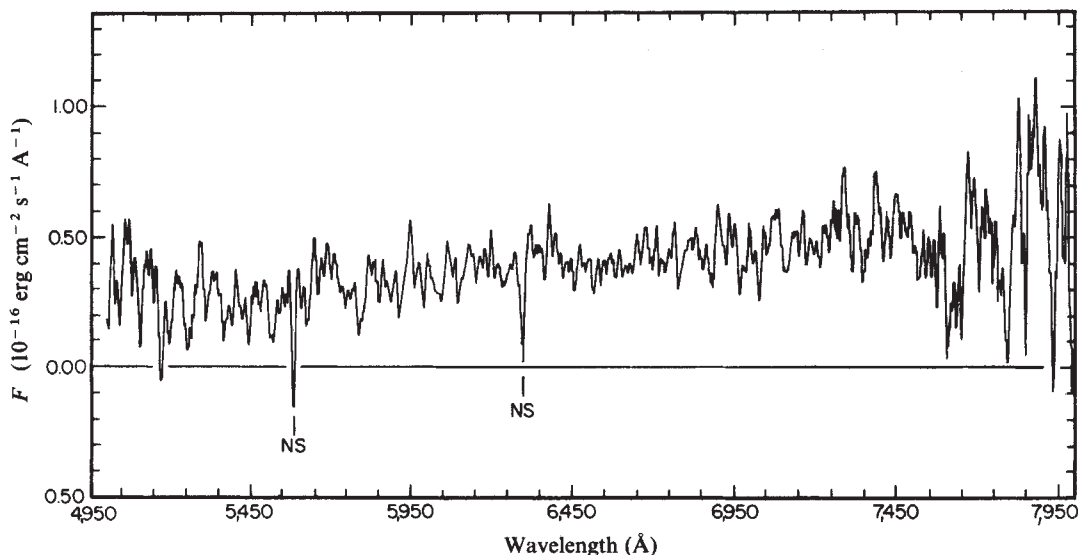


Fig. 1 A spectrum of 0406 + 121 is shown. Only a smoothly rising continuum is apparent, except for two poorly subtracted night sky lines. The spectrum becomes rather degraded shortward of 5,300 Å and longwards of 7,400 Å.

spectral inflection point. First, the IR data of Rieke *et al.* are consistent with $\alpha \approx 2-2.5$, as compared with 3.7 here. Second, if our spectrum is extrapolated, then the predicted K flux is ≈ 10 times greater than a plausible interpolation of the 10 February and 8 March IR measurements. Any outburst this strong on such a short time scale would be unparalleled.

Clearly, further monitoring is essential to establish whether 0406 + 121 and similar objects are simply extreme cases of more familiar sources or represent a truly unique category.

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A dramatic radio outburst in the quasar 1921-29

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We report here an exceedingly large amplitude radio outburst presently occurring in the quasi-stellar source 1921-29 (also known as OV-236). This source which had exhibited slow $\pm 25\%$ variations about a 6 Jy level ($1 \text{ Jy} = 10^{-26} \text{ W m}^{-2} \text{ Hz}^{-1}$) at centimetre wavelengths since 1971 has suddenly increased by more than a factor of 3 to 20 Jy. At 31 GHz, 1921-29 is now the strongest known quasar in the sky, even exceeding 3C273 which has been decreasing since 1975.

Variation curves at four radio frequencies from 1971 to the present are shown in Fig. 1. These observations of 1921-29 are part of our study of the long-term spectral evolution of variable radio sources. The measurements at 89.6 and 31.4 GHz were made with the 36-foot antenna of the National Radio Astronomy Observatory^{1,2} and those at 15.5 and 7.9 GHz were made using the 120-foot antenna of the Haystack Radio Observatory^{3,4}. The sharp rise in flux density begins at 89.6 GHz near 1979.3 and is progressively delayed at lower frequencies. Radio spectra of the source at four different epochs are shown in Fig. 2. The spectral turnover below 15 GHz at 1979.5 and 1979.8 is characteristic of self-absorption in a source emitting synchrotron radiation. The broad spectral maxima imply an inhomogeneous distribution of radiating particles and magnetic field.

The recent outburst is itself superimposed on a more slowly varying component which increased gradually through 1976 and 1977 and then remained constant until early 1979. Its broad flat spectrum in Fig. 2 is also characteristic of a self-absorbed inhomogeneous source. The high frequency transparent portion of the spectrum has a slope of $\alpha \approx -0.4$ above 31 GHz. Like the current 1979 outburst, the rise during 1977 of this slow component was self-absorbed and delayed at lower frequencies.

The spectrum of the rapid 1979 outburst at different epochs can be obtained (Fig. 3) by subtracting the spectrum of the source in 1978, just before the recent outburst. We see that the outburst itself has an initially steep low frequency cut-off with

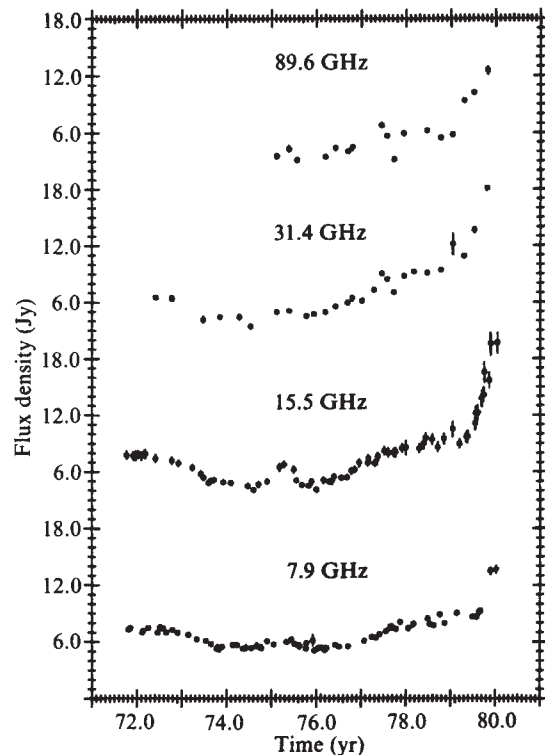


Fig. 1 The observed variation of 1921-29 at four radio frequencies.

spectral index $\alpha \approx +2$ below 15 GHz. As the outburst evolves its low frequency spectrum flattens as the frequency of maximum decreases from about 100 to 30 GHz. The high frequency spectrum above 31 GHz becomes transparent with $\alpha \approx -0.3$, implying $\gamma = 1 - 2\alpha = 1.6$ in a relativistic electron distribution of the form $N(E) dE \propto E^{-\gamma} dE$. Such a hard electron distribution is characteristic of most variable radio sources. This distribution seems to be preserved over the different phase of activity in the source demonstrating the consistency of the particle acceleration mechanism.

The spectral evolution of both the slow 1977 outburst and the rapid 1979 outburst suggest that both are expanding from an initially smaller volume⁵. The initially sharp and eventually broad low frequency turnover of the slow outburst shows that an initially homogeneous component became inhomogeneous as it expanded. The pronounced difference in time scales of the two events must be due either to different rates of expansion or to time compression if there is relativistic motion near the line of sight.

Even though it does not have a published redshift, 1921-29 is undoubtedly a quasar. It has been identified as a stellar object

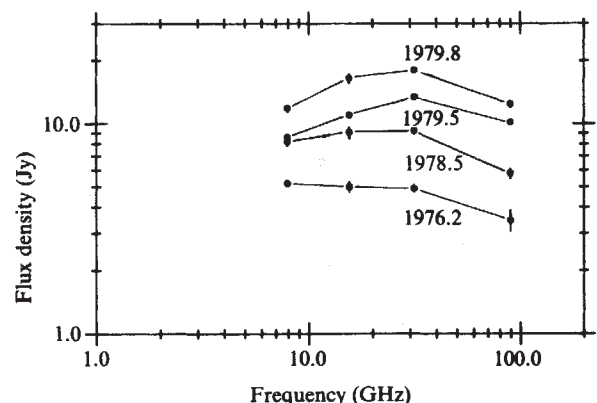


Fig. 2 The total radio spectrum of 1921-29 at four epochs.

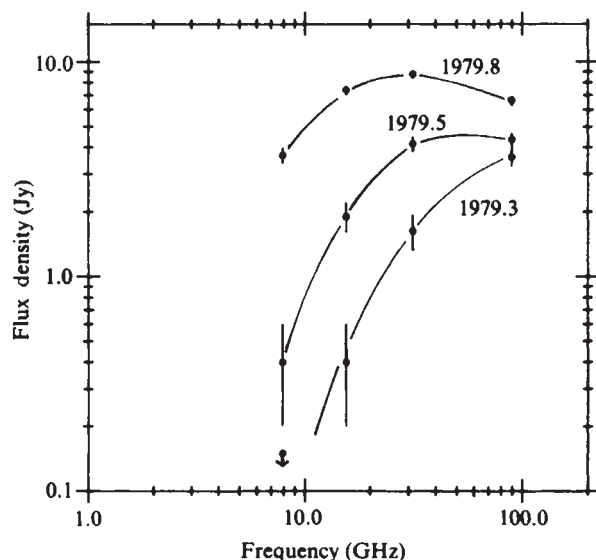


Fig. 3 The spectrum of the 1979 outburst at three epochs obtained by subtracting the average 1978 radio spectrum during 1978.

with a red colour⁶ and flat radio spectrum⁷; it is variable at both radio and optical frequencies⁸ and has angular structure $<10^{-3}$ arc s at 13 cm (ref. 9). The optical position of this 17 mag quasar is 19 h 21 min 41.42 s and $-29^{\circ}20'25.3''$. Optical and X-ray observations of its spectrum and variability during the current outburst would be very valuable.

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Thermal history, chemical composition and relationship of comets to the origin of life

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It is generally believed that a comet consists basically of a loose conglomeration of frozen gases with embedded material similar to that found in the carbonaceous chondritic meteorites, and consequently that comets may be nearly pristine samples of the original solar nebula¹⁻⁵. We show here that thermal processing within comets could have played an important part in determining their present state; in particular, we find that liquid water might have been available in some comets over geologically and biologically significant spans of time. It follows that a cometary origin is not excluded for some thermally metamorphosed meteorites and asteroids, that comets may contain quite complex organic molecules, and that comets may have played a role in the origin and conceivably even in the subsequent evolution of terrestrial life.

Given a mixture of ices and chondritic material with the normal abundance of radioactive elements (U, Th, and K), Lewis⁶ and Whipple and Stefanik⁷ have shown that the central temperature in comets will not exceed the melting point of H₂O. Recently, however, inclusions with anomalous isotopic abundances have been found in certain meteorites which suggest that a nearby supernova explosion may have injected significant amounts of the short-lived radionuclide ²⁶Al into the condensing solar nebula⁸. In fact, the necessity for such a short-lived heat source was earlier implied from studies of the mineralogy of meteorites^{9,10}. As it seems likely that comets formed in the outer portion of the Solar System, at least some of these objects may have received a particularly large dose of such 'extra-solar' material. An important parameter affecting the subsequent thermal history of a comet is the size of the cometary nucleus. Direct observations are largely non-existent, but present evidence is consistent with a model in which at least some new comets have radii of the order of 10 km (refs 11-14), while occasional cometary nuclei could be an order of magnitude or more larger (the largest presently observable possibly cometary object may be Chiron¹⁵, whose radius is probably ~ 100 km (E. Bowell, personal communication)).

Will the surface temperature of a comet heated by such short-lived radioactivity be cool enough to retain the volatile material observed? An upper limit to the surface temperature can be obtained by assuming a sufficiently high thermal conductivity that all internally generated heat is immediately radiated away. The abundance of ²⁶Al in the meteoritic inclusions^{8,16} is ²⁶Al/²⁷Al $\sim 5 \times 10^{-5}$, which yields an available heat of 2 kcal g⁻¹. Since the half-life of ²⁶Al is 7×10^5 yr, we find that heat is generated at a rate $Q = 2 \times 10^{-3}$ erg g⁻¹ s⁻¹. The corresponding surface temperature T_s for a cometary nucleus of density ρ and radius R is $T_s \leq (QR\rho/3\sigma)^{1/4} \leq 120$ K for $R \leq 100$ km, $\rho = 2$ g cm⁻³, and σ = the Stefan-Boltzmann constant. As this is an upper limit and T_s will decrease as the heat source decays, H₂O is certainly retained by the comet¹⁷; and H₂O may control the vaporisation of more volatile compounds, if these are trapped as clathrates in the H₂O lattice¹⁸.

The temperature is potentially quite different in the interior of a comet. The abundance of ²⁶Al is sufficient to melt silicates, let alone icy material, provided thermal loss to the surface of the body is sufficiently slow¹⁶ and provided (as we here assume) that the formation time of comets is not long compared with the ²⁶Al half life. Thus, even if significantly diluted by material without such short-lived radionuclides, sufficient energy would be available to raise the interior temperature of a comet to several hundred degrees. The time scale t_{int} for cooling the central regions is approximately $t_{int} \approx R^2 \rho C_p / k = R^2 / \kappa$ where k is the thermal conductivity and $\kappa = k / \rho C_p$ is the thermal diffusivity. At $T = 120$ K (appropriate to some intermediate region in the mantle of the comet) the thermal constants for ice are $C_p = 10^7$ and $k = 6 \times 10^5$ in c.g.s. units^{19,20}, so that the thermal diffusivity is $\kappa = 3 \times 10^{-2}$ cm² s⁻¹ for $\rho = 2$ g cm⁻³. For a loosely compacted body, however, κ might be significantly lower; for the Moon κ varies from $\sim 10^{-4}$ cm² s⁻¹ near the surface to $\sim 10^{-3}$ cm² s⁻¹ in the deeper regolith^{21,22}. Counteracting the latter effect might be the possibility of thermal transport by gaseous diffusion and convection. If we take the intermediate value $\kappa = 0.004$ cm² s⁻¹, we find $t_{int} \approx 10^9$ yr for $R = 100$ km. For the more typical radius 10 km for a new comet, we find a cooling time for the interior of the order of 10^7 yr. These times might in fact be significantly lengthened by the accretion of an insulating dust layer, as Wood has proposed for the parent planetesimals of certain meteorite classes²³.

Note the following astronomical consequences. Some comets may have undergone significant thermal processing, so that the apparent exposure of, for example, chondritic meteorites and probably some Apollo asteroids to temperatures of several hundred degrees²⁴ does not in itself exclude their being derived from comets. Such an origin has been supported on dynamical²⁵ and spectroscopic²⁶ grounds, although other problems remain²⁷. (If, instead of being initially homogeneous, comets were formed

by accretion of volatiles onto an asteroid core^{28,29}, a similar generic relationship could exist.) Likewise, the existence in new comets of a particularly volatile outer shell, suggested by the appearance of a gaseous coma at large distances from the Sun³⁰, is a natural consequence, although this does not require as strong a heat source as considered here⁷. The possibility of thermal processing must be considered in calculating the chemical composition of the cometary volatiles. The original composition of the nucleus, like that of the Sun and the interstellar medium, will be reducing. Heating such a mixture, particularly in the presence of the metallic and clay catalysts expected to be present, results in the production of organic molecules, including amino acids and nucleic acid bases³¹⁻³³. Complex organic molecules are also produced in such mixtures by ionising radiation, which would result from the radioactive decay postulated here³⁴.

Could comets be relevant to the origin of life? A principal problem in the synthesis of biologically significant molecules in the aqueous terrestrial environment is the polymerisation of amino acids and nucleic acid monomers. Dehydration reactions involving volcanic heating and/or evaporation and catalysis in tidal pools have been invoked^{32,35}. Suitable environments might more naturally occur in a comet, where we may suppose temperature regimes from considerably above the boiling point of H₂O to a perhaps substantially depressed (by dissolved salts) freezing point, solid clay and metallic surfaces for catalysis, possible circulation of an aqueous solution with dissolved organics, and much less dilution than would be expected in a terrestrial ocean. Moreover, the time span determined above during which liquid water might be available in some comets is consistent with geochemical evidence for the epoch of the first O₂-releasing photosynthesis on Earth³⁶. The biological requirement for free energy in a dark environment has been considered by Anders³⁷ in a discussion, similar to the above, concerning the possibility that asteroids might have supported life (we note also the existence of apparently photosynthesis-independent biocommunities at mid-ocean thermal vents³⁸). Present models postulate that the Sun is surrounded by a vast cloud containing perhaps 10¹¹–10¹² comets³⁹ and thus providing environments for a vast number of 'biological experiments'. When a comet enters the inner Solar System, it leaves behind a trail of debris which is encountered by the Earth as meteor showers and micrometeoroids. The Earth accretes about 10⁷ kg yr⁻¹ of such material^{5,40}. Micrometeoroids smaller than 10⁻⁶ g will not be heated above 100 °C on entry, and the interiors of meteorite-sized objects also remain at moderate temperatures^{41,42}. It follows that comets may have played a part in seeding the primitive Earth with biological polymers possibly capable of self-replication, or at least of evolving towards that capability.

Is it conceivable, as suggested by Hoyle and Wickramasinghe^{43,44}, that in subsequent times the Earth has been infected by organic material which arose independently in cometary environments and which was viable in terrestrial organisms? The resemblance between the genetic systems of viruses and cells implies that the evolution of viruses depends on the evolution of cellular genetic systems⁴⁵ and therefore makes the independent evolution of viruses on many different comets very unlikely. On the other hand, it seems difficult to exclude the possibility that small nucleic acids of cometary origin might occasionally be viable in terrestrial organisms if (in the apparent manner of viroids^{46,47}) they could reproduce without the expression of self-specified proteins, so that they need not have evolved a genetic code translatable by the host cell.

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Neutron holography using Fresnel zone plate encoding

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Zone plate shadow casting is a complementary technique to the Leith–Upatnieks modification of Gabor's interferometric method for producing holograms^{1,2}. Suggested originally by Mertz³ in 1961 for use in X-ray astronomy, the technique has received considerable attention in nuclear medicine⁴ for imaging radioactive organs via emitted X rays and γ -rays and, more recently, for examining in a tomographic sense the microscopic spatial source distributions of X rays and charged particles in laser-produced plasmas⁵. A series of simple experiments are described here which demonstrate that zone plate encoded neutron holography can be used to image objects. An object placed in a cold neutron beam from a research reactor scatters neutrons through a Fresnel zone plate, with alternate zones of gadolinium and aluminium, which produces a hologram of the object on an X-ray film. The image is constructed by placing the hologram, linearly reduced in size, in a converging laser beam which produces a real image of the original object. Results are presented for two objects, a pair of 0.01 m diameter rubber spheres and a 0.04 m square Maltese cross made from polytetrafluoroethylene (PTFE).

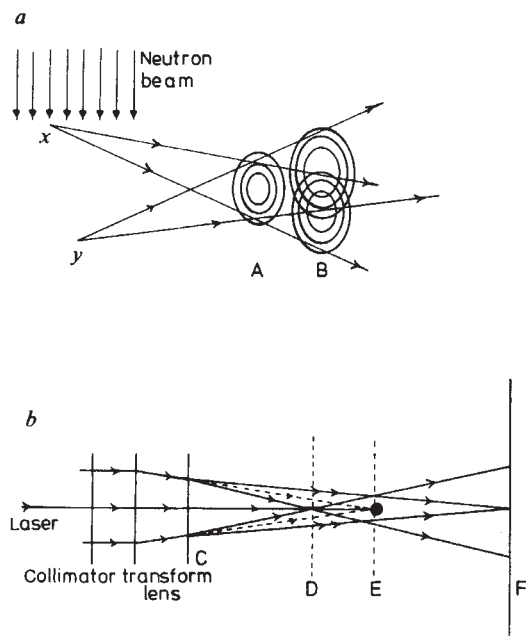


Fig. 1 *a*, Schematic arrangement of two point sources at x and y producing, through a fresnel zone plate at A , a zone plate hologram in a plane at B . The direction of the neutron beam is parallel to the zone plate at A . *b*, The optical arrangement for the reconstruction at E of the object encoded in the hologram at C .

The basic principles of zone plate encoded holography are outlined in Fig. 1. If we consider (Fig. 1*a*) two point sources of radiation at x and y , a Fresnel zone plate at position A will produce in a plane at B two overlapping zone plate shadowgraphs, provided that the wavelength of the radiation from x and y is sufficiently small not to be diffracted by the zone plate. Any solid object, regarded as a linear superposition of points, will produce on a suitable recording material in a plane B a two dimensional hologram consisting of a linear superposition of zone plate shadowgraphs in which is encoded non-coherently the full three dimensional information of the object. The reconstruction of the original object may be achieved coherently by using the optical properties of a Fresnel zone plate, as shown in Fig. 1*b*, which illustrates what is essentially a hardware analogue Fourier transform of the hologram. Each single zone plate in the hologram, acting as a lens, will produce a unique point image of the object point which produced the shadowgraph zone plate. A converging coherent light beam, produced by the transform lens, passes through a linearly reduced transparency of the hologram at a position C . The undiffracted component of the beam is stopped at E . The image at D , due to the negative focal length of the hologram, is superimposed as background on the desired image at E which is due to the hologram's positive focal length. The image in a particular plane through F is the reconstruction of a particular plane in the original object parallel to the original zone plate (Fig. 1*a*). All such object planes may now be retrieved by recording the images on the F -planes spanning the space in which the real image appears.

Neutron holograms have been made for several objects using the 6H beam tube on the DIDO research reactor at AERE, Harwell. This facility provides a well-collimated beam of cold neutrons (energies < 5 meV) with a very low γ -ray contamination. A description of this facility is given elsewhere⁶. As it is those neutrons which are scattered off the object into the zone plate which form the hologram, as illustrated in Fig. 1*a*, it is necessary to work in as large a neutron flux as possible. Consequently all the experiments were carried out inside the reactor shell wall at a distance of 2 m from the reactor face where foil activation measurements gave a value for the neutron flux strength of 3.3×10^{11} neutrons $\text{m}^{-2}\text{s}^{-1}$.

The Fresnel zone plate was made by photographically etching a zone plate pattern ($r_n = r_1 \sqrt{n}$, $n = 1, 2, 3, \dots$) onto a 1.2-mm thick aluminium alloy sheet to a depth of 100 μm and filling the etched zones with a paint containing gadolinium. The aluminium has a low scattering cross-section and is more than 99% transparent to cold neutrons, whilst the gadolinium is essentially opaque. The plate had 47 zones with a first-zone radius (r_1) of 1.12×10^{-2} m. The holograms were recorded on $0.18 \text{ m} \times 0.24 \text{ m}$ X-ray films with a gadolinium foil intensifying screen in front of the film to ensure a linear response in the film emulsion during the relatively long exposure times at these low neutron flux levels. With the recording film 0.4 m from the zone plate which was placed typically at 0.15 m from the objects, with its

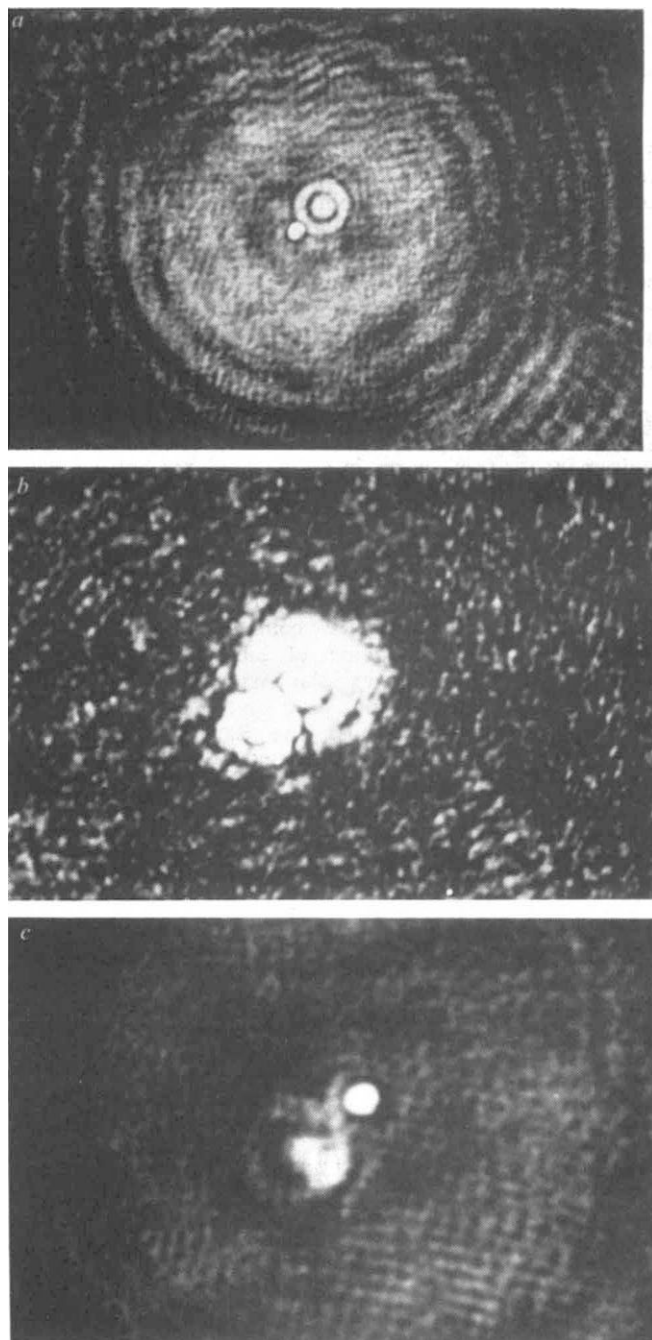


Fig. 2 *a*, A plane through one reconstructed image of sphere (lower left), the other appearing out of focus. *b*, A plane between the spherical images, intersecting neither. *c*, A plane through the second reconstructed image of sphere (upper right), the first image now appearing out of focus.

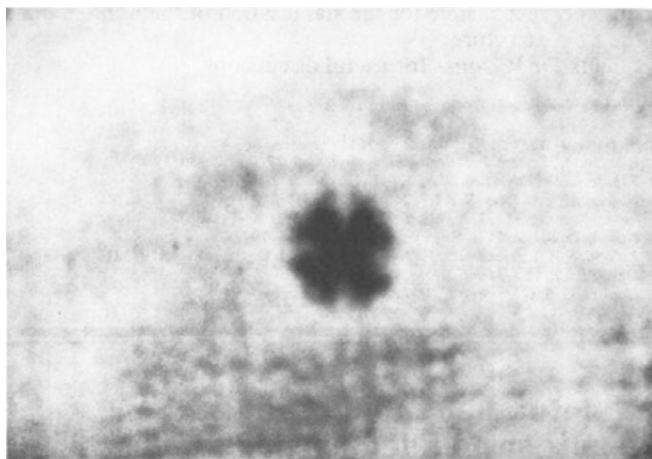


Fig. 3 Optical reconstruction of the Maltese cross.

plane parallel to the beam direction, exposure times using Kodak Industrex C film were 1.5–2 h.

The optical reconstruction was achieved with the arrangement shown in Fig. 1b, with a 2 mW helium–neon laser and a transform lens of focal length 0.15 m. Each neutron hologram was linearly reduced in size by a factor of 40 and a conventional transparency made using Agfa-Gevaert high resolution holographic film type 10E56. Note that a bleaching process recommended for this film, which forms the image by having a desensitised silver halide residue rather than silver grains, can produce reconstructed images with considerably enhanced intensity.

We illustrate the effectiveness of the technique using results for two encoded objects of markedly different geometry. The first test object consisted of two rubber spheres, diameter 0.01 m, suspended in the neutron beam with a horizontal and vertical separation of 0.04 m. The second object was a Maltese cross cut from a 0.04 m square of PTFE of thickness 4 mm. The cross was placed with its plane at an angle of 10° to the plane of the zone plate. A series of planes through the reconstructed images have been recorded on standard fine grain photographic film. Figure 2 shows three such planes through the spatially-separated spherical images of the two original spheres. Figure 2a is a plane through the first sphere at a distance of one-half radius from the surface; the second sphere appears out of focus. Figure 2b is a plane midway between the spherical images and intersecting neither; both images appear out of focus. Figure 2c is a plane through the centre of the second spherical image, the first now appearing out of focus. Finally in Fig. 3 we show a plane through the reconstructed image of our PTFE Maltese cross. Figures 2 and 3 are based on image sizes of about 1 mm. Practical applications of this technique would require larger image sizes which could be achieved by using a telescope in the optical reconstruction system of Fig. 1b. A more detailed technical discussion of this work, including a description of computer-aided reconstructions of the geometry of the images and an assessment of the limits of resolution of the technique, will be published elsewhere.

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Why noble metals favour the face-centred cubic structure

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The noble metals (copper, silver and gold) crystallise in the face-centred cubic (f.c.c.) structure but attempts to calculate their cohesive energy¹ and stacking fault energies² predict that the stable structure is the hexagonal close packed (h.c.p.). By considering a collinear three-ion interaction I have identified a source of energy, of the correct sign and order of magnitude, that explains the stability of the observed structure. Its success is largely due to a distinctive difference in number and spatial disposition of close-packed lines of atoms in the two structures. The principal parameters of the calculation are the scattering phase shifts of the ions and it is therefore highly probable that three-ion interactions also play a significant part in the stability of the transition metals.

The contributions usually considered in studies of metallic cohesion and stability are the Ewald energy, the core overlap energy, the kinetic, exchange and correlation energies of free electrons, and a band-structure energy that is calculated to second order in perturbation theory. Applying such an approach to the noble metals shows that of the two close-packed structures, the hexagonal one is to be preferred because $\Delta E = E_{fcc} - E_{hcp} \approx 0.01$ to 0.003 eV per atom (ref. 1). A related study of stacking fault energies², whose principal considerations are the structure-dependent terms in the energy, is also unsuccessful: it predicts positive energies for the h.c.p. structure and negative ones for the f.c.c. Although minor adjustments or refinements to the usual theory might reverse the sign of the calculated ΔE , I believe that the f.c.c. stability is caused by three-ion interactions and that only these can provide the substantial $\Delta E \approx -0.01$ to -0.03 eV per atom needed to explain the measured stacking fault energies.

This idea is supported by Krause³; he examined a subtle effect in the lattice dynamics of copper and showed that an interplanar stiffness constant derived from $\langle 111 \rangle T$ phonon dispersion data was incompatible with an exclusively two-body potential.

In a study of multi-ion interactions⁴ in simple metals Harrison has drawn attention to a three-ion term in which the ions are collinear and separated by nearest neighbour distances. This is a dominant term because it involves the matrix element for forward scattering and therefore mimics a second order term in

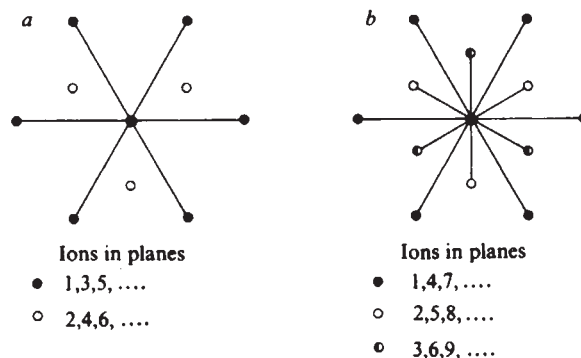


Fig. 1 The disposition of linear triads of ions in the h.c.p. and f.c.c. structures. The projections of ion positions are shown looking down; a, the $[00.1]$ axis in the h.c.p. structure; and b, the $[111]$ axis in the f.c.c. structure.

Table 1 The contribution of the dominant three-ion term to the energy difference between f.c.c. and h.c.p. forms of the noble metals

	Copper			Silver	Gold
E_F^* (eV)	6.80	7.48	8.16	5.58	5.58
$\delta(\pi)$	0.009	-0.027	-0.076	-0.072	-0.265
$\delta(0)$	0.748	2.418	2.969	1.260	1.109
ψ	0.766	2.364	2.817	1.116	0.580
$ t(2k_F) $ (eV)	2.30	2.84	3.41	1.55	1.97
V_0 (eV)	22.1	30.5	40.3	12.2	19.7
Φ_3 (eV per triad)	-0.007	-0.018	-0.015	-0.006	-0.010
ΔE_3 (eV per atom)	-0.022	-0.053	-0.044	-0.019	-0.030

* The free electron values of E_F are 7.00, 5.48 and 5.51 eV respectively.

magnitude. To avoid a complex analysis only this term will be discussed below.

Each atom in an h.c.p. structure is the central atom of three triads which are confined to the close-packed plane (Fig. 1). Each atom in an f.c.c. structure is the central atom of six triads, three lying in the close-packed plane and three spanning three such planes. The net result is a contribution to the energy of the f.c.c. structure that differs from that of the h.c.p. structure by $\Delta E_3 = 3\Phi_3$ where the energy of a single triad is given by

$$\Phi_3 = V_0 8\pi Z \cos(4k_F d + \psi) / (4k_F d)^4 \quad (1)$$

This expression, derived from Lloyd and Oglesby⁵, departs from Harrison's pseudopotential treatment in favour of a form based on scattering phase shifts and, therefore, more appropriate to the noble metals. d is the nearest neighbour distance and $(4k_F d)^3 = 192\sqrt{2}\pi^2 Z$, where Z is the valence (unity in the calculation that follows) and $V_0 = 9\pi Z^2 |t(2k_F)|^2 / E_F$ where $t(2k_F)$ is the t -matrix for on-Fermi-surface back scattering and E_F is the Fermi energy. The phase ψ and $|t(2k_F)|$ are derived from the η_i , the scattering phase shifts for an ion evaluated at the Fermi energy, and are given by

$$\psi = 2\delta(\pi) + \delta(0)$$

and

$$|t(2k_F)| = 4E_F [(\alpha(\pi))^2 + (\beta(\pi))^2]^{1/2} / 3\pi Z$$

where $\cot \delta(\theta) = \alpha(\theta) / \beta(\theta)$,

$$\alpha(\theta) = \sum_l (2l+1) \sin \eta_l \cos \eta_l P_l(\cos \theta)$$

and

$$\beta(\theta) = \sum_l (2l+1) \sin^2 \eta_l P_l(\cos \theta)$$

In the limit of small perturbation $|t(2k_F)| \rightarrow v(2k_F)$ and $\delta(\pi) \rightarrow 0$. Because the perturbation is not small, $|t(2k_F)| / E_F > 0.28$ in the present case, the phase shift method is being used. The data required for the evaluation of equation (1) can be found elsewhere^{6,7} in work in which a precise parameterisation of the Fermi surface is given in terms of four phase shifts. Here the Fermi energy is a free parameter and three sets of results for copper have been given⁶. Table 1 contains the parameters derived from these works and the resulting ΔE_3 , which ranges from -0.02 to -0.05 eV per atom, and may be compared with the experimental estimates⁸ of the intrinsic stacking fault energy: 0.019, 0.010 and 0.022 eV per atom in the fault plane for Cu, Ag and Au respectively. As the contribution of three-ion interactions to the intrinsic stacking fault energy is $-2\Delta E_3$ (ref. 9) it is clear that while these results are in the correct sequence they are, nevertheless, too large. Some reduction in the value of ΔE_3 is to be expected in a full three-ion calculation but a change of sign is highly unlikely. This is because the numbers of the smallest triangular configurations are very similar in f.c.c. and h.c.p. structures. Both have 24 equilateral configurations and 12 configurations with sides in the ratio 1:1: $\sqrt{2}$, and neither of these sets can provide an energy difference between the structures. I conclude therefore, that three-ion interactions are

exclusively responsible for the stabilisation of the noble metals in the f.c.c. structure.

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¹⁵N natural abundance in oceanic suspended particulate matter

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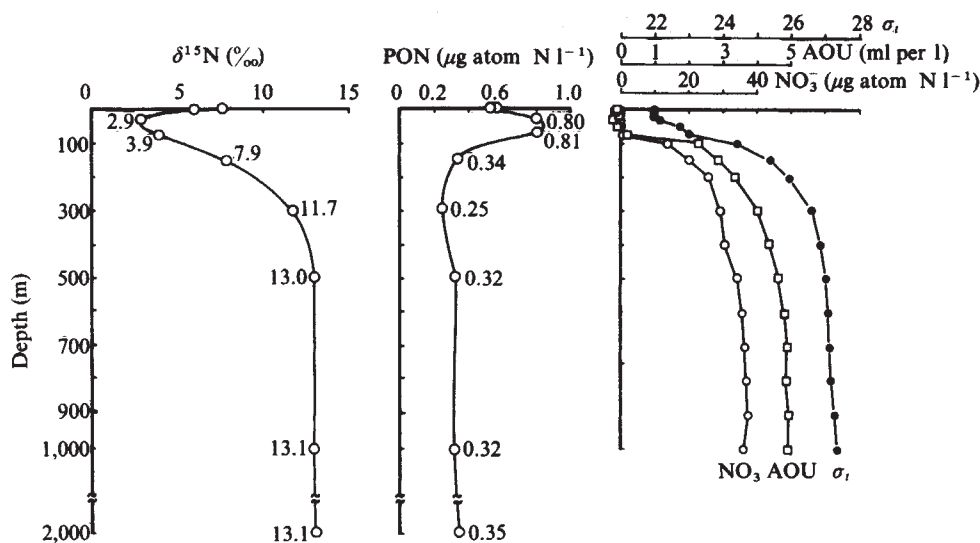
Particulate organic matter (POM) has a central role in the vertical transport of material in the sea¹. In the open ocean, POM is produced in the euphotic layer by phytoplankton and degraded in the aphotic layer during sinking to the sea floor. Isotopic abundance of biophilic elements such as C and N in POM is altered by isotopic fractionations associated with biochemical reactions². Natural abundances of ¹³C or ¹⁵N thus provide useful information on biochemical behaviour of POM in the sea. We report here the first comprehensive data on the vertical distribution of ¹⁵N in suspended POM³ collected at a station in the northeastern Indian Ocean. Also, we discuss how nitrogen isotopic analysis could be used for the identification and quantification of the vertical transport processes.

Williams and Gordon⁴ have investigated ¹³C abundances in suspended particulate organic carbon (POC) collected from the eastern North Pacific, between 10 and 2,940 m, the $\delta^{13}\text{C}$ values fell in the narrow range -22.0 to -24.3 ‰. They suggested that most of the suspended POC is biologically refractory. A variation of $\delta^{13}\text{C}$ in POC with depth was later demonstrated by Eadie and Jeffrey⁵; POC in deeper layers (>330 m) was significantly lower in ¹³C than that in shallower layers. C/N ratios of suspended POM increase with depth⁶. This implies that nitrogen is more rapidly lost than carbon during degradation of suspended POM. Therefore, a much greater variation of ¹⁵N abundance in suspended particulate organic nitrogen (PON) is expected than in ¹³C in POC.

Seawater samples were collected with Van Dorn bottles (25 l) from various depths or with a plastic bucket from the sea surface on Cruise KH-76-5 of Hakuho Maru, University of Tokyo⁷. 20-23 l of seawater was filtered through a precombusted (400 °C, for 1 h) Reeve Angel 984H glass fibre filter (47 mm diameter). The POM collected on the filters was desiccated over silica gel *in vacuo*. The procedures for conversion of PON to nitrogen gas and its purification have been described elsewhere⁸. The total nitrogen and its ¹⁵N content were determined simultaneously using a Hitachi RMU-6E mass spectrometer fitted with a double collector and a dual inlet system. Sufficient nitrogen gas was introduced into the mass spectrometer to produce an output signal at $m/e = 28$ of greater than 7 V. Isotopic ratio measurements were repeated at least four times for each sample and averaged. Two sources of reagent grade ammonium sulphate ($\delta^{15}\text{N}$; -6.7 and -1.5 ‰ relative to atmospheric nitrogen) were used as isotopic standards. Reproducibility of each measurement was $\pm 0.3\%$.

Table 1 summarises the $\delta^{15}\text{N}$ values of duplicate samples collected from the sea surface at 12 different locations. Differences in the $\delta^{15}\text{N}$ of the duplicate determinations ranged

Fig. 1 Vertical distribution of $\delta^{15}\text{N}$, PON, σ_t , AOU and NO_3^- at Station 5 in the northeastern Indian Ocean on 11 January, 1977. Water samples for temperature, salinity, dissolved oxygen and nitrate determinations were collected with Nansen bottles equipped with reversing thermometers. Nitrate was measured using a Technicon Autoanalyser II on board the ship. σ_t and AOU are cited from the cruise report⁷.



from 0.3 to 2.5‰, averaged 1.4‰. Heterogeneity in the distribution of POM and in its isotopic composition may partly cause this variation⁸.

Figure 1 presents the vertical distribution of $\delta^{15}\text{N}$ along with PON, σ_t , apparent oxygen utilisation (AOU)⁹ and nitrate at a station located in the northeastern Indian Ocean. $\delta^{15}\text{N}$ decreased near the surface reaching a 2.9‰ minimum at 30 m and then increased through the 30–500 m depth by ~10‰. Below 500 m the $\delta^{15}\text{N}$ remained constant (~13‰). Conversely, PON increased near the surface and attained a maximum between 30 and 75 m. The abundance of ^{15}N in marine organisms has been shown to increase with increasing trophic level¹⁰. The $\delta^{15}\text{N}$ values for PON collected from the euphotic layer (<150 m), where production of POM exceeds its degradation, are similar to those for phytoplankton while the $\delta^{15}\text{N}$ values for PON from depths >300 m are similar to those of zooplankton or fish.

The increase of $\delta^{15}\text{N}$ with depth parallels the increase in σ_t , AOU and nitrate. This suggests that the ^{15}N enrichment in PON with depth results from isotopic fractionation during oxidative degradation of PON. Wada¹¹ has found that $\delta^{15}\text{N}$ increases by 3–4‰ during the early period of oxidative degradation of dead phytoplankton and zooplankton at room temperature and atmospheric pressure. Our results suggest that degradation of PON actively proceeds in the upper 500 m, leaving refractory components which sink into the deep layers.

Low values for $\delta^{15}\text{N}$ in the subsurface PON may result from isotopic fractionation associated with nitrate uptake by phytoplankton in light-limited conditions, because growth of phytoplankton in this subsurface layer is mainly supported by nitrate¹². Preferential utilisation of ^{14}N -nitrate over ^{15}N -nitrate by marine diatoms has been reported¹³. The ^{15}N enrichment of nitrate in eastern Pacific shallow waters over that in deeper waters also supports this view¹⁴.

In the surface layer of the tropical Indian Ocean, the concentrations of nitrate are extremely low, and the growth of phytoplankton is mainly sustained by regenerated ammonia¹⁵. Under these circumstances, isotopic fractionation is minimal and the relatively high $\delta^{15}\text{N}$ values for surface PON can be attributed to a rapid recycling of nitrogen¹⁶.

There are two main mechanisms for the vertical transport of particulate matter in the sea: (1) transportation mediated by slowly sinking fine suspended particles, which are easily collected on filters by filtering small amounts of seawater; and (2) transportation mediated by rapidly sinking larger particles such as faecal material, which are not collected adequately by conventional water sampling³, but can be collected by *in situ* filtration of large seawater volumes¹⁷ or by use of sediment traps^{18,19}.

The $\delta^{15}\text{N}$ values reported for organic nitrogen in marine sediments are 10‰ or less^{10,20}. The $\delta^{15}\text{N}$ values for suspended

PON in deep water is much higher (Fig. 1). If the organic nitrogen in sediments is solely supplied by suspended PON, the lower sediment organic nitrogen $\delta^{15}\text{N}$ values are difficult to explain. A recent survey that we conducted in the northern North Pacific found that PON collected with sediment traps is invariably less enriched with ^{15}N ($\delta^{15}\text{N}$, 2.9–4.4‰) than are sediments, irrespective of the collection depth (1.1–5.25 km). Simultaneous determinations of ^{15}N abundance in suspended PON, settling PON collected with sediment traps, and sedimentary organic nitrogen should provide an estimate of the relative contribution of suspended and settling PON to the organic nitrogen in bottom sediments, and may provide a way of assessing the importance of these processes in the vertical transport of particulate material.

Table 1 $\delta^{15}\text{N}$ and PON at the sea surface

Station	Lat	Long	PON ($\mu\text{g atom N l}^{-1}$)	$\delta^{15}\text{N}$ (‰)	$\Delta\delta^{15}\text{N}$ (‰)
5	10°59.3' S	110°59.4' E	0.57	6.0	1.6
			0.53	7.6	
6	13°57.1' S	103°01.5' E	0.34	6.8	2.2
			0.38	9.0	
7	17°01.5' S	95°00.4' E	0.31	7.3	0.7
			0.32	8.0	
8	20°01.5' S	86°58.6' E	0.41	8.3	0.3
			0.63	8.6	
10	10°00.4' S	86°58.9' E	0.40	5.1	1.0
			0.59	6.1	
12	0°03.5' N	86°56.1' E	0.41	8.5	
13	5°02.2' N	86°58.1' E	0.44	3.2	0.9
			0.50	4.1	
15	14°03.5' N	86°58.1' E	0.51	7.6	2.5
			0.57	10.1	
17	5°02.8' N	107°29.0' E	0.74	3.8	2.3
			0.81	6.1	
18	8°59.7' N	110°28.5' E	0.98	6.0	1.3
			0.89	7.3	
19	12°58.8' N	113°29.1' E	0.62	5.5	2.5
				8.0	
20	17°03.0' N	118°12.0' E	0.60	5.2	0.3
			0.66	5.5	
21	20°03.2' N	120°30.4' E	1.37	2.1	1.4
			1.10	3.5	
Mean				1.4 ± 0.8	

^{15}N abundance is presented in terms of $\delta^{15}\text{N}$ as defined by the equation:

$$\delta^{15}\text{N} = \frac{(^{15}\text{N}/^{14}\text{N})_{\text{sample}} - (^{15}\text{N}/^{14}\text{N})_{\text{standard}}}{(^{15}\text{N}/^{14}\text{N})_{\text{standard}}} \times 10^3$$

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Pyroclastic sulphur eruption at Poás volcano, Costa Rica

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The recent Voyager missions to Jupiter have highlighted the role of sulphur in volcanic processes on Io^{1–7}. Although fumarolic sulphur and SO₂ gas are almost universal in terrestrial active volcanoes, and rare instances of sulphur lava flows have been reported^{8,9}, sulphur in a pyroclastic form has only been described from Poás volcano, Costa Rica¹⁰. Here we amplify the original descriptions by Bennett and Raccichini¹⁰ and describe a recent eruption of pyroclastic sulphur scoria and ejected blocks that are characterised by miniature sulphur stalactites and stalagmites.

Poás volcano is a small composite volcano (height 2,700 m, basal diameter 20 km) with a deep central crater 1.5 km wide and 300 m deep, in the floor of which is a smaller crater 400 m in diameter containing a hot (~50 °C) crater lake. Intermittent eruptions take place from one or more sites within the crater lake. These are generally small geyser-like plumes of steam and mud, but are occasionally much more vigorous, ejecting large, altered blocks, and more rarely, fragments of juvenile material. Small eruptions were taking place at intervals of roughly 20 min through much of 1978, but seem to have ceased since September 1978.

Bennett and Raccichini¹⁰ noted the presence of greenish sulphur particles up to 4.5 cm in diameter around the crater in 1977. The particles resemble basaltic scoria in size and shape, and their nature and distribution indicated that they have been erupted as a shower of molten particles. Bennett and Raccichini attributed the 'sulphur scoria' to a 'lake' of molten sulphur

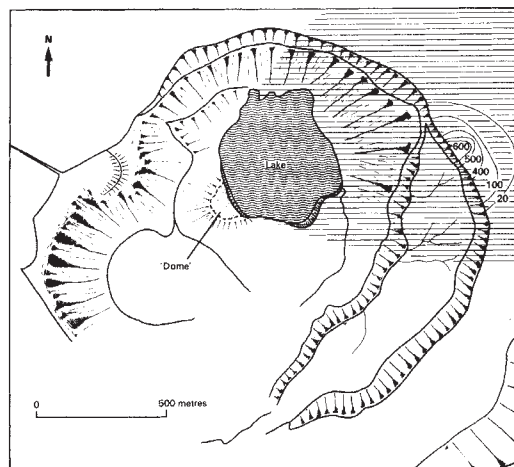


Fig. 1 Map of summit area of Poás volcano. Horizontal lines, area covered by ejected blocks. Numbered contours, surface concentration of sulphur particles in g m⁻².

located beneath the water in the floor of the crater, and estimated that 'several hundred tonnes' of sulphur were lying on the surface around the crater.

We have carried out observations at Poás during January 1978 and February–March 1979. On the former occasion, frequent small eruptions and one large eruption took place; on the latter occasion, no eruptions were observed. In 1978 we were able to confirm Bennett and Raccichini's descriptions of scoriaceous sulphur particles dispersed widely, but sparsely, around and within the crater. At that time some of the sulphur was observed lying at, or near, the surface of an andesitic ash erupted in 1953 (ref. 11), and it therefore post-dates that eruption. Much the greatest proportion of the scoriaceous sulphur, however, had been washed downslope and had accumulated locally as deposits many centimetres thick. These deposits were striking on account of their greenish colour, and the shapes of the particles within them, some of which resembled Pele's tears which are characteristic of fluid basaltic eruptions¹².

In September 1978 a large eruption took place. Although poorly observed and recorded, due to persistent cloud covering the volcano, rangers of the Costa Rica National Park Service reported hearing loud explosions and seeing small, but widespread, blue flames around the crater rim after the eruption, and that the level of water in the lake had decreased sufficiently for



Fig. 2 Typical small ejected block and impact crater or eruption of September 1978. Note asymmetric position of block on crater rim; direction of travel was from right to left.



Fig. 3 Sulphur encrusted face of an ejected block, showing stalactites of sulphur. Stalactite and stalagmite nearly connect at right. The coin is 2.1 cm in diameter.

the bottom to be exposed in places. Our visit in February 1979 confirmed that at least one powerful explosive event had taken place, as the eastern flanks of the volcano were littered with fresh impact craters and large ejected blocks (Fig. 1). New fragments of green scoriaceous sulphur were abundant on the eastern rim of the crater, locally sufficient to cover the surface entirely. The distribution of the sulphur was studied by weighting the amounts found within individual 1-m square grids, and constructing a contoured map (Fig. 1). The greatest concentration of sulphur is on the east rim of the crater, whereas most of the older ashes have accumulated on the north and northwestern flanks, in response to the prevailing winds. This indicates that the sulphur particles were deposited by a directed blast, rather than falling from a wind-blown eruption cloud. A total of ~4 tonnes of pyroclastic sulphur particles lies on the surface examined, which suggests a total for the eruption of some 10–15 tonnes, allowing for sulphur which fell within the crater itself. Grain size analysis of the sulphur shows that, in size distribution and sorting characteristics, it resembles the basaltic ash of 1953 (unpublished data).

The asymmetrical distribution of the impact craters and ejected blocks from the September 1978 eruption also indicates a directed blast (Fig. 1). Blocks 0.3 m in diameter were found occupying craters 1.5 m in diameter several hundred metres from the crater rim. In most cases, the trajectories followed by the blocks were nearly tangential to the gently sloping flanks of the volcano and many blocks were therefore found lying at one side of their craters, or had bounded out of their initial craters and travelled onwards (Fig. 2). Measurement of the final resting places of the blocks relative to their impact craters enabled the azimuths of their trajectories to be estimated and the approximate site of initial ejection to be located. This corresponds closely with a breached dome on the crater floor, which is also the site of continuous intense fumarolic activity, and is heavily encrusted with fumarolic sulphur deposits.

Examination of the blocks revealed three distinct types to be present: (1) poorly consolidated lumps of grey clay and mud which had clearly been derived from the floor of the crater lake; (2) blocks of fresh, dark basaltic material which may represent the juvenile component involved in the eruption; and (3) heavily altered, sulphur encrusted blocks probably derived from the site of the active fumaroles. The sulphur encrusted blocks are concentrated towards the centre of the bombed zone, which corresponds closely to the area of maximum pyroclastic sulphur deposition. The distribution of the pyroclastic material is consistent with a powerful explosion having taken place at the foot of the cliffs at the present fumarole site, so that most of the ejecta were blasted out towards the east. A similar explosion may have taken place in 1964, as Krushensky and Escalante¹³

described sulphur encrusted blocks in the ejecta from that eruption.

The unique aspect of the blocks ejected in 1978 is illustrated in Fig. 3. After coming to rest, the molent sulphur on the exterior surfaces of the ejected blocks dribbled downwards, dripping off projecting points to form stalactites, and accumulating on the surface beneath as stalagmites a few centimetres high. The formation of these features after the blocks came to rest is indicated by their vertical orientations, and by the formation of stalagmites on the loose ash and gravel of the preexisting surface. In a few cases, blocks were found exhibiting stalactitic features which were not vertical. These may have been produced during heating of the blocks before the eruption.

In contrast to the green scoriaceous sulphur resting on the surrounding surfaces, the sulphur encrusting the ejected blocks is strikingly yellow in colour. The difference in colour may represent an original difference in temperature of formation, or rate of cooling. An impression of the physical state of the yellow sulphur during stalactite formation is afforded by the fact that each stalagmite is formed of 20–30 individual pancake-like blobs of sulphur piled on top of each other and fused together. Clearly, the sulphur drips were at or near the melting point of sulphur, but the liquid sulphur was also of low viscosity.

As Sagan emphasised⁵, the viscosity of liquid sulphur at its melting point is ~0.1 P but increases by a factor of 10^3 to a value exceeding 10^2 P between 157 and 187°C. Thus while the sulphur on the ejected blocks cannot have greatly exceeded the melting point (110–119°C), the green scoriaceous sulphur may have achieved temperatures of up to 200°C, because the morphology and grain size characteristics of the fragments (unpublished data) are strikingly similar to those of true basaltic scoria formed from liquids with viscosities between 10^3 and 10^4 P (for example, data summarised in ref. 14).

Although it is curious that they have not been described before, the small stalagmites and stalactites on the ejected blocks seem to have a relatively straightforward origin. During the week before the large eruption of January 1978, we observed a marked increase (~10°C) in the temperature of the fumaroles in the dome on the crater floor. The highest temperatures recorded were well above the melting point of sulphur. Presumably, a similar temperature rise preceded the eruption of September 1978, and was sufficient to melt the sulphur contained in a large volume of rocks surrounding the fumaroles. The molten sulphur accumulated in joints and fractures in the rock until the explosive eruption took place, and broke up the original mass into a large number of smaller blocks, and hurled them across the crater. Liquid sulphur still adhering to the joint faces of the blocks was partially stripped off in flight, but sufficient still remained on impact to dribble to the ground, forming stalactites and stalagmites.

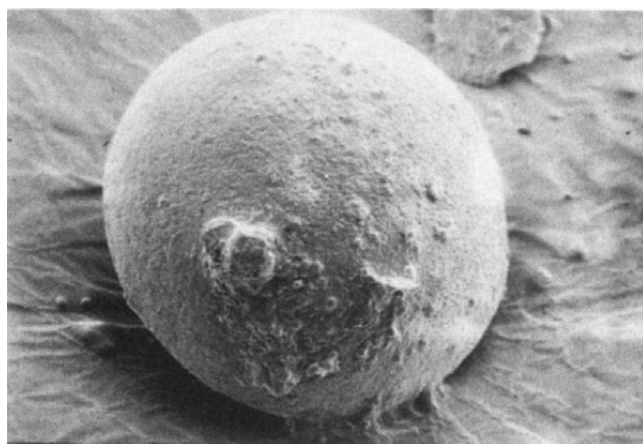


Fig. 4 Scanning electron micrograph of sulphur sphere from Poás lake mud. Sphere is 500 μ m in diameter.

The scoriaceous sulphur is more difficult to account for. The 10–15 tonnes erupted in 1978 must have had a similar origin to that described by Bennett and Raccichini. They, however, reported the ejection of 'several hundred tonnes' of sulphur¹⁰, which may be the products of several similar eruptions over a period of time.

We consider it unlikely that a 'lake' of liquid sulphur existed within the crater, as proposed by Bennett and Raccichini, but it seems probable that liquid sulphur congregated in fissures and pore spaces in the volcanic conduit beneath the site of the active fumaroles, and that this was sprayed into the air during the explosive eruption of September 1978.

The presence of water in the crater lake seems to have played an important part in this unusual eruption. Initially, it was responsible for sealing off the liquid sulphur from contact with the air, thus preventing its combustion or oxidation. Subsequently, interaction of the molten sulphur with lake water may have been responsible for the vigour of the eruption, and for the shaping of some of the sulphur particles. Evidence for this is the presence, within the lake muds and floating on the lake surface, of myriads of tiny spheres of sulphur (Fig. 4), which may have been produced when a spray of liquid sulphur was blasted into the lake water from beneath. We do not consider that temperatures as high as the boiling point of sulphur (444 °C) suggested by Bennett and Raccichini are necessary to explain the observed phenomena, particularly as the viscosity of liquid sulphur decreases between 200 and 444 °C (see ref. 5).

Although sulphur has an important role in terrestrial volcanism, it is clearly dominant in ionian volcanic processes^{1–7}. On Earth, sulphur and sulphur gases are an important component of explosive volcanic activity¹⁵ and commonly form up to 30% of volcanic gases¹⁶ which are removed from the atmosphere by surface processes. However, sulphur lavas and pyroclastic rocks as described here from Poás are relatively rare. On Io, sulphur dioxide is the dominant gas in the sustained volcanic plumes and sulphur may be a major component of the ionian crust^{4,5}. Assuming that both have approximately chondritic Fe:S:Si ratios, the difference in surface sulphur concentration between Earth and Io presumably lies in their internal structures. Assuming the presence of a massive Fe–FeS core in the Earth¹⁷ the magmatic source regions for terrestrial volcanic rocks must be relatively depleted in sulphur. On Io, by contrast, vigorous internal convection driven by tidal dissipation¹⁸ might have been responsible for less efficient core formation and for less efficient partitioning of sulphur into its putative core.

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Rb–Sr age and source of the Bimodal Suite of the Ancient Gneiss Complex, Swaziland

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The Ancient Gneiss Complex (AGC) in Swaziland^{1–5} is a complexly deformed suite of gneisses that may be divided into three major units²: (1) the Bimodal Suite (BMS) of inter-layered siliceous low-K leucocratic orthogneisses and amphibolites; (2) homogeneous tonalitic gneiss (the ~3,320 Myr old Tsawela gneiss)^{6,7}; and (3) supracrustal rocks constituting two main lithologic sequences—the Mkhondo Valley^{1,5} and Dwalile (ref. 8 and M.P.A.J. and A.C.W. unpublished data) metamorphic suites. The Tsawela gneiss is intrusive into both the BMS and the Dwalile metamorphic suite⁸. Relating the BMS to the greenstone sequence of the Barberton Mountain Land (the Onverwacht Group of the Swaziland Supergroup) has caused much controversy. It has been thought¹ that the BMS predates the Onverwacht Group and may represent a basement to the latter. Another view^{9,10} is that all of the leucocratic gneisses of the AGC are younger than the Onverwacht Group and were derived from an unrelated mantle source. The amphibolite and metasedimentary rocks in the AGC are xenoliths of the Swaziland Supergroup. This controversy has remained unsolved due to the lack both of unambiguous contact relationships between the AGC and the Swaziland Supergroup, and of reliable radiometric data on the emplacement age of either unit. At the only known locality where these two units are juxtaposed, the contact is tectonically deformed. Early radiometric age data^{6,11–20} have given results that are either too imprecise or reflect subsequent metamorphism⁷. Recently, however, a precise age of $3,510 \pm 60$ Myr (2σ) has been obtained by the Sm–Nd technique for extrusion of the volcanic rocks of the Komati Formation of the lower Onverwacht Group²¹. The results are reported here of Rb–Sr whole rock and biotite–whole rock isotopic analyses of samples of the leucocratic orthogneisses of the BMS. Results are also presented of initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio studies of samples of amphibolite from the Komati Formation. The relationship of the AGC to the Swaziland Supergroup is re-examined. All Rb–Sr ages mentioned are calculated using $1.42 \times 10^{-11} \text{ yr}^{-1}$ as the decay constant of ^{87}Rb .

The BMS has been intruded by the ~3,320 Myr old Tsawela gneiss^{6,7}, the ~3,030 Myr old Lochiel (Homogeneous Hood) granite (refs 15, 22 and J.M.B., unpublished data) and mafic dykes. Faulting and mylonitisation in some areas have intensely deformed the BMS gneisses and fresh exposures of the BMS commonly look like smooth pavements. It is, therefore, difficult to obtain unaltered samples of the BMS in the type locality near Mankayane (Fig. 1). However, a quarry has recently been opened near the Fairview Dam in north-central Swaziland (Fig. 1) where the BMS is largely free from younger pegmatitic veins but is intruded by two generations of basic dykes. Vein-free samples of the leucocratic orthogneisses of the BMS were collected in this quarry at least 50 m from the dykes.

The analytical techniques used are described elsewhere^{23,24}; the results are summarised in Table 1. Regressing the results of whole rock analyses of the leucocratic orthogneisses of the BMS²⁵ yields an isochron ($\text{SUMS}/(n-2) = \text{MSWD} = 0.31 <$

Table 1 Analytical data

Sample	Rock unit	Rb (p.p.m.)	Sr (p.p.m.)	$^{87}\text{Rb}/^{86}\text{Sr}^*$	$^{87}\text{Sr}/^{86}\text{Sr}^\dagger$	$^{87}\text{Sr}/^{86}\text{Sr}^\ddagger$
B-79-8A	BMS of AGC	77.3	256.	0.8596	0.7441	
B-79-8B	BMS of AGC	86.0	262.	0.9357	0.7483	
B-79-8B (bio§)	BMS of AGC	642.0	32.5	72.73	3.755	
B-79-8C	BMS of AGC	72.7	274.	0.7539	0.7389	
B-79-8D	BMS of AGC	97.9	222.	1.2592	0.7653	
B-79-8E	BMS of AGC	88.6	275.	0.9185	0.7475	
B-79-8F	BMS of AGC	97.3	365.	0.7583	0.7390	
B-79-8G	BMS of AGC	86.4	273.	0.9021	0.7466	
B-79-8H	BMS of AGC	71.3	272.	0.7468	0.7388	
B-79-8I	BMS of AGC	71.9	292.	0.7003	0.7363	
B-79-8J	BMS of AGC	86.7	267.	0.9274	0.7479	
B-76-40	Komati Fm.	1.167	26.3	0.1259	0.7064	0.7000
B-76-41	Komati Fm.	1.751	33.7	0.1473	0.7077	0.7002
B-78-19A	Komati Fm.	1.116	31.3	0.1011	0.7052	0.7000
B-78-19B	Komati Fm.	1.192	9.44	0.3589	0.7186	0.7003
B-78-19C	Komati Fm.	0.758	5.87	0.3674	0.7185	0.6997
B-78-19D	Komati Fm.	1.877	159.5	0.0344	0.7014	0.6996
AB9 ¹¹	Komati Fm.			0.0311	0.7016	0.7000
AB9 ^{1A}	Komati Fm.	0.272	40.9	0.0192	0.7014	0.7004
V36A ¹	Komati Fm.			0.0505	0.7023	0.6997
V36A ^{1A}	Komati Fm.	0.334	37.9	0.0255	0.7019	0.7006
AU20-37 ^{1A}	Komati Fm.	0.882	20.0	0.127	0.7064	0.6999
VU32A ^{1A}	Komati Fm.	0.690	9.06	0.220	0.7115	0.7003

* Uncertainty = 1.4% (2 σ)† Uncertainty = 0.02% (2 σ)

‡ Calculated for 3,510 Myr.

§ Biotite. The biotite-whole rock age was calculated from sample B-79-8B.

¹¹ Ref. 18. ^{1A} Ref. 19.

2.5) (ref. 26) of $3,555 \pm 111$ Myr (2 σ) with an initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.6999 ± 0.0016 (2 σ) (Fig. 2) (MSWD, mean square of weighted deviates). A biotite-whole rock age of $\sim 2,889$ Myr was also obtained.

The very low initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of the whole rock isochron suggests that the calculated age corresponds closely to the time of emplacement of these rocks. This age is indistinguishable from the $\sim 3,510$ Myr age²¹ of emplacement of the volcanic rocks of the Komati Formation, implying that these two units are radiometrically coeval. The leucocratic orthogneisses of the BMS, at least where sampled, cannot be a much older basement to the Onverwacht Group of an age similar, for example, to the $> \sim 3,790$ Myr old Sand River Gneisses of the Limpopo Mobile Belt²⁷. The biotite-whole rock age implies that the major deformations manifested in the rocks of the BMS occurred by $\sim 2,890$ Myr ago. In view of their closeness, the major deformations displayed in the rocks of the Swaziland Supergroup, broad open and recumbent isoclinal folding²⁸, had also probably taken place by this time.

Unaltered samples of volcanic rocks from the Swaziland Supergroup have not yet been collected and all of the samples analysed so far have been metamorphosed to at least greenschist facies. The lack of deformation fabrics in many of these samples suggests that their mineral composition is not due to regional metamorphism in the conventional sense, but more probably to deuteric alteration. In addition, because no Rb-Sr whole rock isochrons corresponding to an age of $\sim 3,510$ Myr have been generated from samples from the Komati Formation, no direct determination of the initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of these rocks have been made. Part of the scatter in the analytical results may reflect open system behaviour. However, in view of the $\sim 3,430$ Myr mineral isochron age derived from a single sample of the Komati Formation¹⁹, the data scatter more probably reflects a range in initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios established within these rocks before this time. Part of this range may be attributed to chemical changes during deuteric alteration but some may also reflect a genuine range in $^{87}\text{Sr}/^{86}\text{Sr}$ ratios in the magmas themselves. Deuteric alteration generally increases the $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of the rocks. Therefore, the maximum initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of each sample may be estimated by subtracting the radiogenic ^{87}Sr component that would have been generated over 3,510 Myr to

leave the present $^{87}\text{Rb}/^{86}\text{Sr}$ ratio from the measured $^{87}\text{Sr}/^{86}\text{Sr}$ ratio. Results from the extrusive rocks of the Komati Formation have been treated this way in Table 1. Isotopic data¹⁹ from sills and other differentiated intrusive bodies in the Komati Formation have been omitted because of uncertainty in their age with respect to the Komati Formation and their obviously altered composition¹⁹. The data from the extrusive rocks yield an average maximum initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of 0.7001 ± 0.0006 (2 σ). It seems, therefore, that the Komati Formation is not only coeval with the leucocratic orthogneisses of the BMS, but that the initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of these units are indistinguishable.

These data are consistent with the possibility that the leucocratic orthogneisses of the BMS and the extrusive rocks of the Komati Formation are genetically related. It has been suggested from REE analyses⁵ that these orthogneisses were derived from partial melting of basalt or amphibolite²⁹, having similar chemical compositions to the extrusive rocks of the Komati Formation. It is possible, therefore, that the rocks of the Komati Formation represent the parent that, on partial melting, yielded the leucocratic component of some of the BMS. The amphibolite in the BMS then could be remnants of the Komati Formation.

However, recent findings in southeastern Swaziland oppose this age relationship. In the western part of the Mankayane type area (Fig. 1), the BMS is in conformable contact with strongly distorted and disrupted metasupracrustal rocks, lithologically akin to the Onverwacht Group, known as the Dwalile metamorphic suite (ref. 8 and M.P.A.J. and A.C.W., unpublished data). These rocks have not been dated but are older than the $\sim 3,320$ Myr old^{6,7} Tsawela gneiss that intrudes them (ref. 8 and M.P.A.J. and A.C.W., unpublished data). The original relationship between the BMS and the Dwalile suite is obscured by repeated episodes of high strain. Nevertheless, the metasupracrustal rocks are commonly preserved in the cores of synforms and no field relationships nor structures indicating that the BMS is younger than the Dwalile suite have been observed. Furthermore, the lower amphibolite grade of the rocks at Dwalile suggests a higher crustal level of metamorphism than that of the rocks of the BMS that are metamorphosed at upper-amphibolite facies (ref. 2 and M.P.A.J. and A.C.W., unpublished data). Available structural and metamorphic

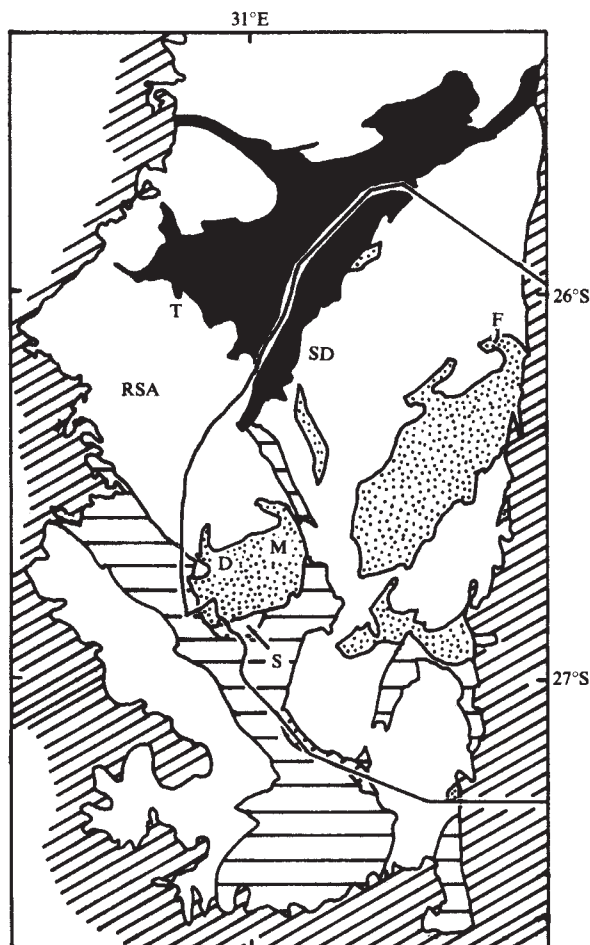


Fig. 1 A simplified geological map of the Barberton Mountain Land and the area to the south modified from Hunter². ■, Swaziland Supergroup. ▨, Ancient Gneiss Complex. □, Younger granitoid intrusions. ▤, Pongola Group and the Ushushwana Complex. ▩, Transvaal and Karoo Supergroups. D, Dwalile; F, Fairview Dam; M, Mankayane; T, Theespruit pluton; S, Sicunusa pluton; SD, Swaziland; RSA, Republic of South Africa.

evidence therefore suggests that a basement–cover relationship exists between the BMS and the Dwalile suite. If this is true, it follows that the parent rocks of the BMS here could be older than the Komati Formation. The rocks classified in the BMS at the type locality may be different from those at the Fairview Quarry.

The initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of the leucocratic orthogneisses of the BMS and those postulated for the Komati Formation are small and plot on the commonly proposed oceanic mantle

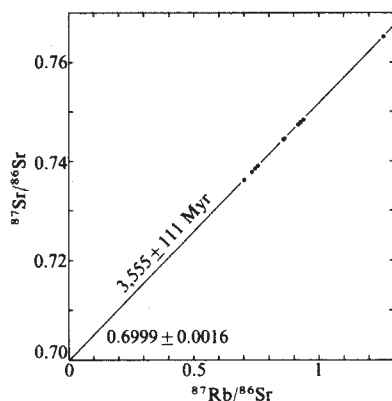


Fig. 2 Isochron diagram of the leucocratic orthogneiss of the BMS of the AGC.

Sr-isotopic growth curves^{30,31}. These curves reflect oceanic mantle depleted in Rb with respect to Sr. Both the leucocratic orthogneisses and the Komati Formation may be derivatives of this type of mantle.

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Mesozoic complementary crust in the North Atlantic

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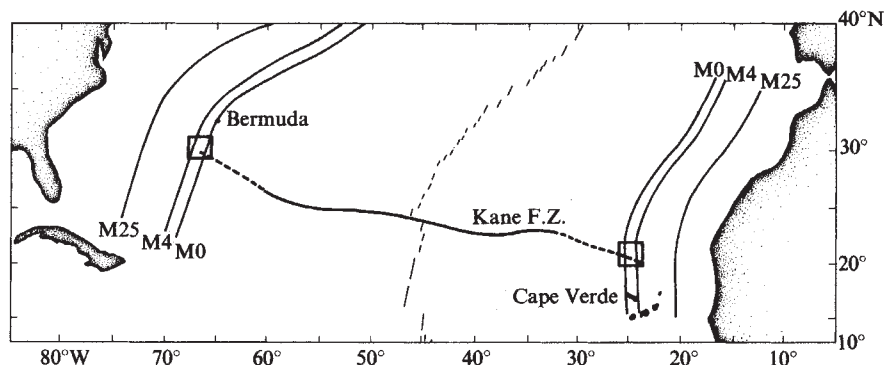
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Detailed surveys of complementary oceanic crustal sections have been carried out over Mesozoic magnetic anomalies M4–M0 (~116–110 Myr BP) in the North Atlantic Ocean. We report here reconstructions of these crustal sections, one on the North American plate and one on the African plate, as they once existed together at the ridge crest using a statistical least squares technique, as well as by eye. The reconstructions allow a test of plate rigidity and consequently a refinement of the reconstruction of the whole North Atlantic in these times. Previous reconstructions had the uncertainty that the correct sections were being matched; the gentle curvature of the Mesozoic plate boundary provides little constraint along the strike of the boundary. The unique local pattern of the magnetic anomalies and their offsets in the surveyed areas allows an unambiguous match.

Fig. 1 Locations of two detailed survey sites.



On the North American plate a detailed geophysical survey of an approximate 1° square south of Bermuda was conducted in 1975 as part of the International Phase of Ocean Drilling¹ (Fig. 1). The additional magnetic data are from RV Atlantis II, RV Chain, USNS Keathley and a Project Magnet aeromagnetic survey²; fracture zone positions were picked from a Project Magnet magnetic anomaly contour map³ on the basis of steep gradients, truncation of linear anomalies, and the presence of localised anomalies (Fig. 2a). The complementary crust on the African plate was surveyed by the Vening Meinesz Laboratory on board the MV Tyro in a 1° by 2° area north of the Cape Verde Islands in 1978 (Fig. 1). Additional magnetic and seismic data were obtained from the HMNS Snellius, RV Atlantic II, RV Vema, RV Conrad, USNS Gibbs, USNS Discoverer, and the USNS Kane. Fracture zone positions on the African plate were determined from seismic reflection data and picked as the deepest part of the trough (Fig. 2b).

The uniqueness of the magnetic pattern confirms that the two data sets are complementary. The survey on the North American plate showed that two left lateral fracture zones exist roughly 75 km apart and that the offsets across the fracture zones changed between M4 and M0 time. On the southern fracture zone the offset increased from ~15 to 35 km, and on the northern fracture zone decreased from ~40 to 30 km. These changes in fracture zone offset were accommodated by asymmetric spreading and rotation of the accretion axis¹. An examination of the western Atlantic magnetic lineation map as

compiled by Schouten and Klitgord⁴ shows that this pattern of M4 and M0 lineations is unique. Finding the same pattern on the African plate, then, proves that the surveys are of complementary pieces of crust. On the African plate two left lateral fracture zones 75 km apart were surveyed; offsets for M4 and M0 were 15 and 35 km on the southern fracture zone and 40 and 30 km on the northern fracture zone (Fig. 2b).

On the African plate the location of the survey was predicted by rotating the North American data to Africa (Fig. 3a) by the M0 pole of Schouten and Klitgord (personal communication). This 'initial' Schouten and Klitgord pole is based solely on shipboard magnetic anomaly positions, the data set consists of ~60 anomaly identifications for each plate, spread over 23° in the central North Atlantic. In this reconstruction, the trends of the anomalies match quite well, but the predicted fracture zone positions fall roughly 20 miles south of the surveyed positions.

Poles of rotation which bring the estimated transform faults into better alignment were calculated for the two detailed data sets. Considering the density of data it seemed reasonable to use the least squares method of Hellinger⁵ which gives a statistical best fit. Poles were also calculated from a visual fit to allow a comparison of methods.

The least squares method takes into account the estimated error for each data point; a computer program searches for the best fitting pole and angle of rotation within a specified area and range. The data are split into segments of spreading ridges and transform faults; data in a given segment from each plate are

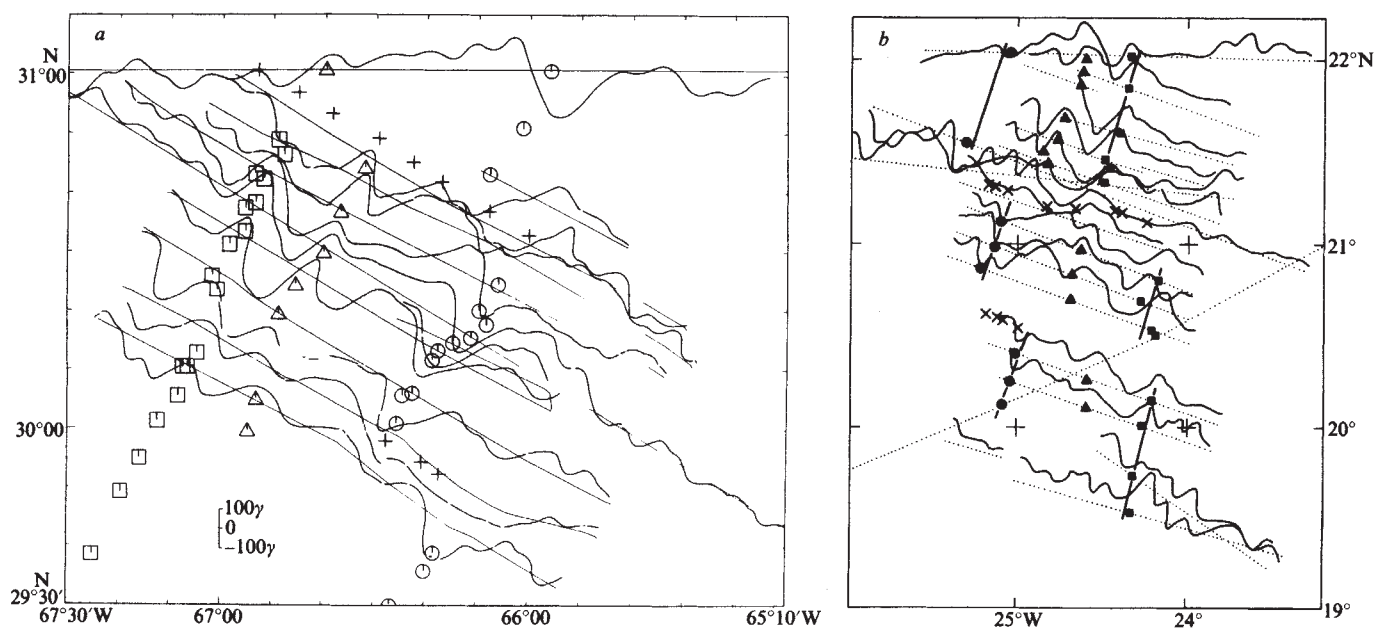


Fig. 2 Magnetic anomalies plotted along track with anomaly positions and fracture zone positions (+, x) superimposed. a, North American plate; b, African plate. ●, M0; ▲, M2; ■, M4.

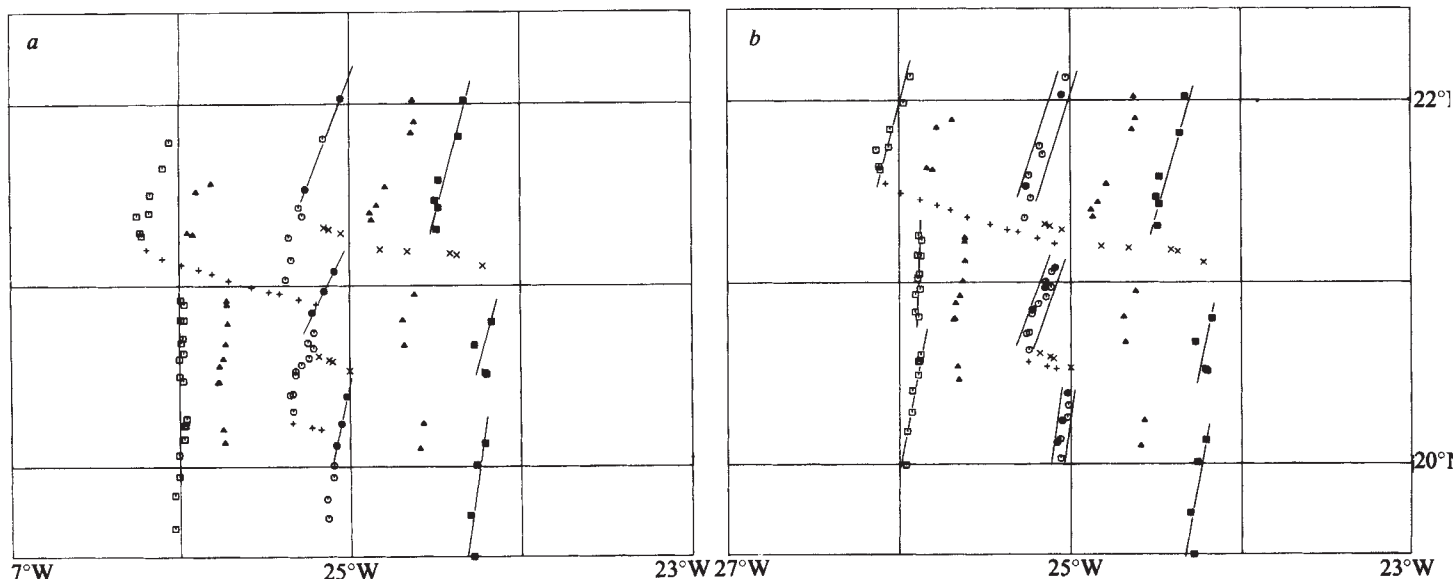


Fig. 3 *a*, Reconstruction of relative plate positions using the initial Schouten and Klitgord pole of rotation for M0. *b*, Reconstruction of relative plate positions using the least squares pole of rotation for M0. ○, M0; △, M2; □, M4.

matched. The data are rotated by a given pole and angle, and a great circle is computed for each segment of the plate boundary by a least squares technique. The closer the data fall on the great circle, the better the fit is considered to be.

Goodness of fit is measured by calculating the distance of a point from its great circle, squaring this quantity, and summing for all data points and all segments. This sum is called the fit measure and is computed for poles and angles within the specified ranges. The pole and angle with the smallest value of the fit measure is considered the best. Points known more accurately are preferentially weighted.

For these computations, magnetic anomalies were assigned an error in location of ± 3 km, and transform faults ± 10 km. The relative magnitudes of the errors are the important factor in the computations. The position of the transform fault active at a specific time within a fracture zone was considered to be less well constrained than the position of the magnetic anomaly isochrons. Uncertainties in ship navigation and variability in magnetic anomaly shape are such that the error in magnetic anomaly location is probably more realistically estimated as 6 km. Poles of rotation computed using an error of 10 km for the magnetic anomaly positions differed by a few tenths of a degree from those using an error of 3 km.

The data for magnetic anomaly M0 were divided into three ridge segments and two transform fault segments. The pole of rotation which gives the best fitting reconstruction of the M0 plate boundary is 66.78°N , 19.00°W with a rotation angle of 53.75° (Fig. 3*b*). We will call this the 'least squares pole'. The pole calculated from a visual reconstruction of M0 is 66.71°N , 19.25°W with a rotation angle of 53.85° (reconstruction not shown). (We call this the 'visual pole'.) In the visual fit fracture zone positions were made to be more coincident than on the rotation by the least squares pole. This condition is not necessarily valid as the North American transform fault positions were picked from magnetic anomalies and the African from seismic reflection profiles. The actual pole positions, however, are almost identical.

The M0 least squares pole of rotation and its contoured fit measure have been plotted (Fig. 4) along with the positions of the visual pole and the initial Schouten and Klitgord pole of rotation. The smallest fit measure for each pole on a 1° grid was plotted and contoured. A trough strikes roughly north-east-south-west containing poles with a low value of the fit measure, that is, poles that give 'good' or statistically acceptable fits. The 'best' pole lies within a local minimum.

Data for M4 were treated similarly (Table 1).

The poles calculated from the detailed data set for M0 and M4

by the least squares technique and the visual techniques are within $\frac{1}{2}^\circ$ or 1° of each other. The least squares technique has the advantage of allowing a computation of the fit measure, a numerical description of how good each computed reconstruction is.

Both poles as calculated only from the detailed data sets were applied to the regional data and gave good fits for the whole ocean basin. The least squares pole gave a better fit than the visual pole. As a study of the fit of 2° of plate boundary has given a good description of the fit of 23° of plate boundary, the two plates must have behaved with a high degree of rigidity.

Schouten and Klitgord have since considered the detailed data and recomputed the M0 pole which also fits the regional

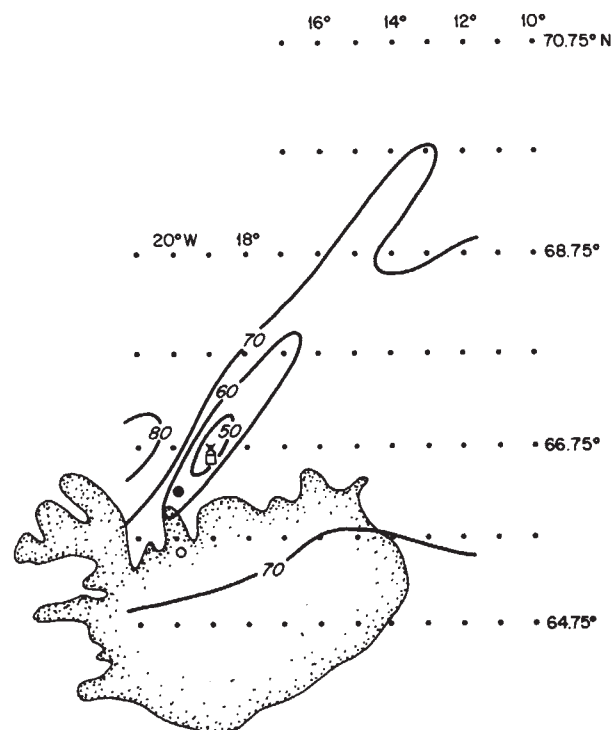


Fig. 4 Measure of fit for M0. The initial Schouten and Klitgord (○), the least squares (×), the visual (□), and the refined Schouten and Klitgord (●), pole of rotation positions have been plotted.

Table 1 Poles of rotation

	M0			M4		
	Lat	Long	Rotation angle	Lat	Long	Rotation angle
Initial Schouten and Klitgord	65.6	-19.9	54.71	65.3	-19.6	57.18
Least squares	66.75	-19.00	53.78	65.25	-21.00	57.55
Visual	66.71	-19.25	53.85	64.74	-21.55	58.11
Refined Schouten and Klitgord	66.3	-19.9	54.37	66.1	-19.0	56.40

magnetic data. This 'refined pole of rotation' lies 1° south-west of the least squares pole; it gives a statistically and visually satisfactory fit for the detailed data set. This suggests that poles of rotation based on widely scattered data can be improved by studies of small areas with a dense data coverage. This study further shows that in regions of sparse data, poles of rotation may be reliably calculated from detailed surveys of small areas of complementary crust.

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Is a black coat in the desert a means of saving metabolic energy?

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Black goats of small body size (15-22 kg) dominate the herds of the Bedouins inhabiting the extreme deserts of the Middle East; only a few white goats appear in such herds. Annual rainfall in these deserts varies from 15 to less than 150 mm; resources of food are sparse and watering points widely spaced. Summer is extremely hot and winter may be bitterly cold. Cloudy days are rare and solar radiation is immense all the year round. Presumably, special adaptations would have been required in order to thrive in these harsh deserts. Can the black colour of the Bedouin goat be considered such an adaptation? Here, we have attempted to answer this question, and have found that by absorbing more of the Sun's energy on cold days, the temperature of the coat dramatically increased and the goat's oxygen consumption decreased significantly.

Short-wave radiation has been shown experimentally to penetrate animal coats by multiple diffuse reflection. For white coats, short-wave radiation may be scattered into the coat and absorbed below the surface, facilitating the inward flow of heat to the skin. For black coats, short-wave radiation is absorbed near or at the fur surface; little is reflected but a large amount is re-radiated as long-wave radiation before it reaches the skin^{3,4}.

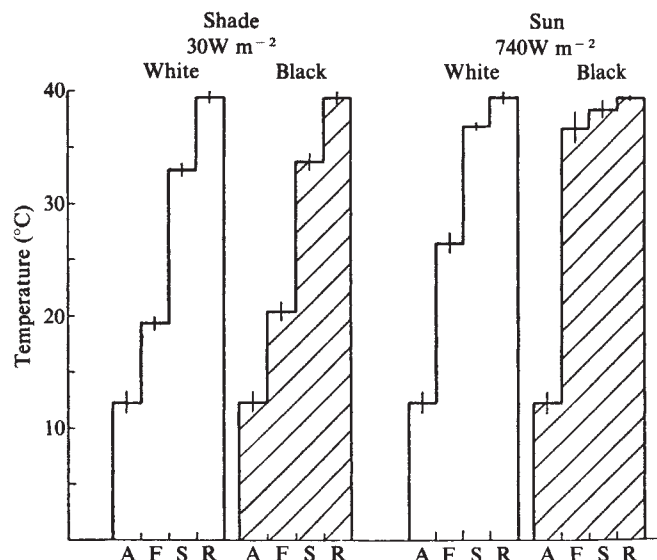


Fig. 1 Ambient and body temperature of two black and two white Bedouin goats, as measured during a clear winter day in the desert. Experimental time 0930 to 1430 h. Values are mean $\pm$ s.e. (vertical bars) of 35 measurements. A, Air; F, coat; S, skin; R, rectal.

This suggests that the black coat may thus reduce the heat gain and the need for evaporative cooling. However, recent studies have negated this possibility. It has been shown that the black goat absorbs about 80% of the solar radiation impinging on its fur and evaporates appreciable amounts of water, mainly through its skin⁵⁻⁷. In contrast, white goats absorb only 40% of the impinging radiation, and, consequently, evaporate much less water⁶. Black colour in the Bedouin goats is therefore not advantageous during the hot desert summer. The purpose of the present study was to determine whether their black coat enables the goats to improve their energy balance during the desert winter, when energy demands of the goat are high but food availability is at a minimum⁸.

To assess the contribution of coat colour to the energy economy of the goats, we set out to answer two questions: (1) what is the effect of solar radiation on body temperatures and metabolism of Bedouin goats during a typical winter day in the desert, and (2) is this effect the same in black and white goats? Experiments were carried out during the winters (mid-December to mid-February) of 1976-8 in the mountainous region of the Sinai Desert. Two black, mature females (18.5 kg body weight) were purchased from local Bedouins. To ensure acclimatisation, they remained in their original herd for the entire year, except for the experimental period (12 measurement days altogether), when they were taken to the nearby study area. In addition, two local white goats were studied. They were also kept in the same herd as the black ones. Oxygen consumption, rectal, coat surface and skin temperatures of the goats were measured simultaneously, as the goats stood quietly either fully

Table 1 Oxygen consumption of black and white Bedouin goats during a clear winter day in the desert

	Oxygen consumption (ml O ₂ per kg per h)		Metabolic reduction (%)
	Shade	Sun	
Black goat	488 ± 20	351 ± 28	28.1
White goat	488 ± 18	436 ± 17	10.7

Values are mean $\pm$ s.e. of 35 measurements. Radiation in the shade 30 W m⁻², radiation in the Sun 740 W m⁻². Air temperature in both experimental conditions was 12.2 °C.

Table 2 Changes in coat temperature and metabolism of black and white goats, as a result of transfer from shade to Sun

	Change in metabolism* (ml O ₂ per kg per h)	Change in coat temperature† (°C)	Rate of metabolic change (ml O ₂ per kg per h per °C)
Black goat	137	16.8	8.1
White goat	52	6.9	7.5

Air temperature in both experimental conditions was 12.2 °C.

* From Table 1.

† From Fig. 1.

exposed to the Sun or in the shade. The techniques and their accuracies were the same as those described in detail elsewhere^{5,6}, except that in the present study the animal and air temperatures were measured by fine (36-g) copper-constantan thermocouples. The measurements were made between 0930 and 1430 h, when air temperature (mean 12.2 °C, s.d. 3.5 °C) and solar radiation (mean 740 W m⁻², s.d. 95 W m⁻²) were fairly stable. All the measurements were made over 30-min intervals; a period of 45–60 min was allowed to ensure thermal equilibrium before each experiment.

No difference in either body temperature or metabolic rate was found between black and white goats as long as they stood in the shade. However, when exposed to the Sun, the coat and skin temperatures of both black and white goats increased and their metabolic rate decreased. These changes were far greater in the black goats (Fig. 1, Table 1). As air temperature was the same in both Sun and shade, it is clear that the change in oxygen consumption depends on the change of the gradient between core body to coat surface temperature. The change in oxygen consumption per degree of temperature change is much the same in both goats (Table 2). This indicates that the differently coloured coats have similar insulative characteristics. Therefore, the larger metabolic reduction shown by the black goat is due to a greater change in its coat surface temperature, resulting from a higher absorption of the solar radiation.

To assess the role of black colour in a desert animal, one should take into consideration the entire climatic cycle to which the animal in question is exposed. We propose here that the critical season of the year for the Bedouin goat is the relatively short, cold desert winter and not the hot, dry summer. Presumably, water is not the crucial selection factor, because this breed is equipped with specialised physiological mechanisms which enable the goat to withstand prolonged periods of water deprivation^{9,10}. On the other hand, these goats are not equipped with efficient mechanisms to prevent heat losses when exposed to cold. The loose and shaggy coat of the goat is a poor insulator. This is reflected in the rather high, lower critical temperature (26 °C) obtained for this breed⁹. Therefore, to keep its body temperature constant during cold weather, the goat must rely mostly on metabolic energy rather than on insulation. The reduction in metabolic rate, associated with absorption of more solar radiation by the black coat, can therefore be regarded as a mechanism which may be critical for the goat's survival during periods of food scarcity in winter. It is reasonable to assume, therefore, that selection pressure for survival of the Bedouin goat in the desert has centred on energy economy rather than water economy. The finding that black goats need less energy provides a logical explanation for the seeming paradox that the Bedouins have selected black animals for their milk and meat producers in hot deserts.

Black robes of goat's hair are commonly worn by Bedouins in the desert. A recent study carried out during the hot desert summer has demonstrated that the effect of a black robe on the heat balance of man is the same as the effect of a white one¹¹. However, in the light of our measurements, it would be interesting to determine whether during winter in the desert, the black robe has a role similar to that we assign to the black fur of the goat.

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Role of the surface carbohydrates in sperm-egg interaction in *Ciona intestinalis*

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In the ascidian *Ciona intestinalis*, species-specific recognition and binding between spermatozoa and eggs occurs at the outer surface of the vitelline coat (the chorion). Attachment of spermatozoa to the chorion occurs via very thin, Ruthenium red-positive fibrils connecting the plasma membrane of the tip of the spermatozoon with the fibrillar tufts of the outer surface of the chorion¹. Experiments with fluorescein-conjugated lectins suggest that glucose or mannose and fucose residues are exposed at the surface both of the chorion and of the spermatozoon, while *N*-acetyl-glucosamine is only present at the surface of the chorion². Work presently underway shows that the prevailing sugar components of the chorion are fucose, glucose and *N*-acetyl-galactosamine. However, this last sugar does not seem to be exposed at the surface of the chorion². The role of the sugar residues exposed at the surfaces of the chorion and spermatozoon in gamete recognition and binding has been investigated to determine whether these sugars are functional components of the gamete receptors. It would be expected that binding of the spermatozoa to the chorion and fertilisation should be competitively inhibited in the presence of excess fucose, glucose and/or mannose, while *N*-acetyl-galactosamine and *N*-acetyl-glucosamine should not be effective. Our present results suggest that fucose residues play a key part in the binding reaction and in fertilisation.

For a quantitative estimate of the binding reaction we have used 'glycerol-treated' eggs (ref. 3). We have previously shown^{1,3} that treatment of unfertilised eggs with glycerol does not impair the species-specific binding of the spermatozoa to the chorion. However, in these conditions the spermatozoa fail to undergo the acrosome reaction. This has allowed us to dissect out the events of sperm attachment from those of the acrosome reaction and sperm penetration through the chorion¹.

For a quantitative study of the effect of the various sugars on gamete interaction we have used the subtraction method of Vacquier and Payne⁴. The sperm concentration is linearly related to absorbance at 340 nm. The binding was routinely checked in the light microscope under dark field illumination³; this showed uniform distribution of spermatozoa attached to the eggs at all sperm concentrations used. The maximum number of spermatozoa attached to eggs occur between 3 and 6 min after 'insemination' (Fig. 1). The maximum percentage of bound spermatozoa is between 20 and 40% depending on the concentration of free spermatozoa. By varying the concentration of

sperm available per egg, the maximum number of bound spermatozoa can be seen to be reached at a ratio of about 8×10^3 spermatozoa per egg (Fig. 2); in these conditions the estimated number of spermatozoa bound to each egg is about 2,500–3,000. This ratio was used in experiments of competitive inhibition with the sugars.

Preliminary experiments also showed that none of the sugars tested, even at the highest concentrations affect either sperm motility or oxygen uptake (Yasumasu, personal communication). However, the effect of these sugars is completely reversible. The effect of the different sugars on the binding reaction is shown in Fig. 3. Of the sugars tested, L-fucose is proved to be the only one which effectively competed with the binding of the spermatozoa to the chorion. The L-fucose concentration required for maximum inhibition is 50 mM; at 25 mM inhibition is approximately halved; at 10 mM this sugar is completely ineffective. These concentrations may, at a first glance, seem high. However one should consider that at the site of contact between spermatozoon and the receptors on the chorion the concentration of the sugar is likely to be very high. Similarly the interaction between lectins and their receptors is inhibited only at high sugar-lectin ratios.

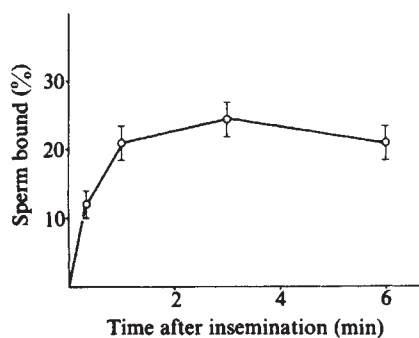


Fig. 1 Samples, each 1 ml, of a 5% suspension of glycerol-treated eggs (corresponding to 5×10^3 eggs) were 'inseminated' in small beakers with 1 ml of sperm suspension at a concentration of about 3.5×10^3 sperm available per egg. At the times indicated, 2 ml of 2% glutaraldehyde in sea water was added (which caused the eggs to sink rapidly to the bottom of the beaker). The absorbance of the supernatant (unbound spermatozoa) was then measured. The number of bound spermatozoa was calculated as the difference between the unbound and the total number of spermatozoa in the original suspension. The mean and standard deviations are calculated from four experiments.

A puzzling observation for which we have no explanation is that when fucose-treated eggs were checked under the microscope there was always about 10% of the eggs whose chorion was covered by attached spermatozoa (those which were not removed by repeated washing³), while the remainder did not have any attached spermatozoa at all. Even though the percentage of these eggs is small it is nevertheless bound to lower the value of the overall effect of fucose on sperm binding. When D-fucose, the uncommon isomer of L-fucose was tested under the same conditions, no effect whatsoever was observed. The results with glucose were variable; in some experiments the inhibition of binding ranged from 60 to 80% while in other experiments there was no effect. Figure 3 also shows that mannose, N-acetyl-glucosamine and N-acetyl-galactosamine have no inhibitory effect. The effect of galactose, although not present either in the chorion or in the sperm plasma membrane was also tested and proved to be completely ineffective.

We have also tested the effect of saccharides on fertilisation. In each experiment samples of 100 eggs from the same animal were suspended in the same concentrations of sugars used for inhibition of binding experiments. After 30 min of incubation the same amount of sperm ($2-6 \times 10^5$ spermatozoa ml^{-1}) was added and 1 h later the eggs were checked for cleavage. The inhibitory effect of the L-fucose on the fertilisation response was

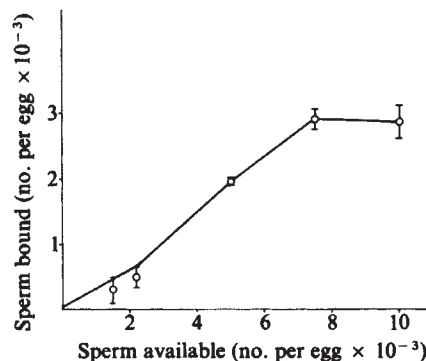


Fig. 2 Experimental conditions were as for Fig. 1. A constant number of eggs ($5 \times 10^3 \text{ ml}^{-1}$) was exposed to different concentrations of spermatozoa for 6 min, whereby the final sperm egg ratio (sperm available per egg) ranged $1.7-10 \times 10^3$. The mean and standard deviations are calculated from four experiments.

proportional to the sugar concentration. In 15 experiments out of 18, 100% inhibition was observed at the highest concentration (50 mM) while at the lowest one (10 mM) there was no difference with respect to the controls. The inhibition ranged from 20 to 50% when the sugar concentration was 25 mM. The effect of glucose was again variable; in the majority of experiments from 40 to 100% of eggs were fertilised while in a few cases none were fertilised. The other sugars tested (mannose, galactose, N-acetylgalactosamine and N-acetylglucosamine) were totally ineffective. The effect of fucose is reversible; indeed binding and fertilisation occur normally when eggs incubated in fucose are then washed and inseminated. In another series of experiments, living eggs were inseminated in the presence of L-fucose; an aliquot was transferred to a dialysis tube and dialysed against sea water for 1 h. The eggs recovered from the dialysis tube proved to be fertilised while the undialysed controls did not cleave. The effect of fucosidase as well as of other glycosidases is now being tested.

The results described here strongly suggest that L-fucose residues present on the plasma membrane of the spermatozoon and/or on the outer surface of the chorion are functionally involved in the recognition between spermatozoa and eggs. Interpretation of the molecular mechanisms involved must await further work. However in view of the presence of the same sugar residues both on the surface of the chorion and on the plasma membrane of the spermatozoon (as suggested by our

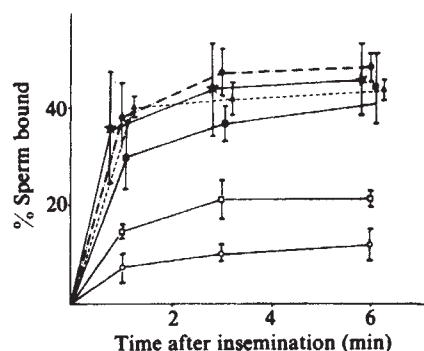


Fig. 3 Eggs ($5 \times 10^3 \text{ ml}^{-1}$) and spermatozoa ($8-9 \times 10^3$ available per egg) were each suspended in sea water containing the sugar at a final concentration of 50 mM (unless otherwise indicated). The two suspensions were then mixed and the results checked at the time indicated in the abscissa. Fucose was additionally used at 25 mM concentration. In each experiment all sugars were tested and control experiments were performed using eggs and spermatozoa from the same stock. (All sugars tested were from Sigma.) The mean and standard deviations are calculated from six experiments. Control (●); fucose (○); fucose 25 mM (□); mannose (▲); N-acetyl-glucosamine (★); N-acetyl-galactosamine (■).

previous work²) a possible model might be the one proposed by Cook⁵ for carbohydrate-carbohydrate interactions in all hydrated phase-containing carbohydrates and Ca^{2+} . The model is based on the observation that fucose and, in fact any uncharged sugar can form sugar- CaBr_2 complexes in which Ca^{2+} is bound to two symmetry-related sugar molecules and to three water molecules, thus resulting in the formation of hydrated fucose- Ca^{2+} -fucose bridges.

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Ca^{2+} -dependent hormonal stimulation of ciliary activity

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It has been demonstrated that calcium modulates ciliary activity in protozoa¹ and also in epithelial cilia of the mussel gill². Changes in the frequency of ciliary beat in the oviduct of the salamander have also been associated with movement of Ca^{2+} across the cell membrane³. However, the control of ciliary activity in mammals is poorly understood and the function of calcium in mammalian cilia and its role in coupling hormonal effects to changes in ciliary activity have been only preliminarily investigated⁴. The evidence presented here suggests that the stimulus-response coupling of the stimulating effect of prostaglandin E_2 (PGE_2) on ciliary activity of the rabbit oviduct is carried out by release of intracellular Ca^{2+} .

To avoid artefacts introduced by uncontrolled changes in the viscosity of epithelial secretions, experiments were conducted in cultures of ciliated cells from the rabbit fimbria. Tissue was grown according to the method of Rumery *et al.*⁵ which yields cultures rich in ciliated cells and almost devoid of secretory cells. The animals were pretreated with a single intravenous (i.v.) dose of 100 μg of luteinising hormone administered 10 h before explanting the tissue. A total of 24 cultures of oviductal ciliated cells were grown in Rose chambers containing Eagle's medium (Steinberger modification) with 10% horse serum, pH 7.5, and incubated for 36-48 h at 37°C.

Ciliary activity was monitored by laser light-scattering spectroscopy. A description of the application and validation of this method in comparison with the measurement of ciliary motion by high-speed cinematography has been published elsewhere⁶.

The cultured cells were first equilibrated in Hanks' solution, pH 7.3 at 37°C for 30 min. The Rose chambers were then positioned in a Reichert inverted microscope modified for laser scattering spectroscopy. The attenuated and collimated beam of a Spectra Physics 124A He-Ne laser was directed at the culture chamber illuminating an area of approximately 8,000 μm^2 containing about 10,000 cilia. The laser light scattered by the moving cilia was collected by the objective lens of the microscope at an angle of 35° from the plane of the chamber. The changes in intensity of the scattered light were detected as current fluctuations by a photomultiplier tube (RCA-C7164R). The spectrum of the photocurrent fluctuations which directly gives the statistical distribution of frequencies of ciliary beat (see Fig. 1 inset), was processed on-line by a fast Fourier transform digital spectrum analyser developed in our own laboratory.

The cultures were first scanned to find clusters of ciliated cells demonstrating a stable and narrow peak of ciliary beat frequen-

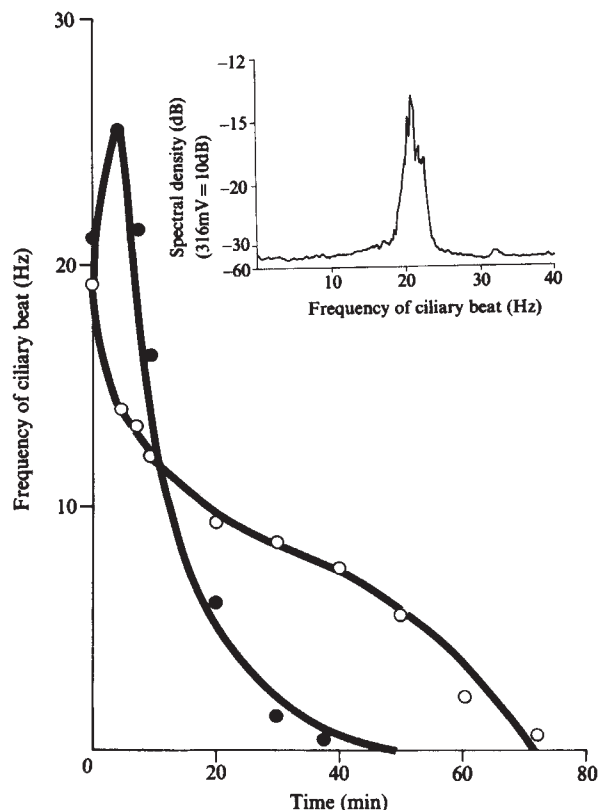


Fig. 1 Typical time course of the effect of decalcification on the frequency of beat of ciliated cells from the rabbit oviduct in tissue cultures. At time zero the cultures were perfused with Ca-free Hanks' solution containing 2 mM EGTA with (●) or without (○) PGE_2 (10^{-7} M). Note the initial increase followed by an exponential decrease in the frequency of ciliary beat produced by PGE_2 . Inset shows the spectrum of ciliary beat obtained by laser scattering spectroscopy. Note the presence of a single dominant peak centred at a frequency of 21 Hz.

cies for at least 30 min. Following this control period, the Rose chamber was perfused with 10 ml of Ca-free Hanks' solution containing 2 mM EGTA. The frequency of ciliary beat was monitored for the next 60-80 min until ciliary arrest was observed (Fig. 1). The cultures were then re-equilibrated in Hanks' solution containing EGTA-buffered calcium concentrations of either 10^{-6} , 10^{-5} , or 10^{-3} M Ca^{2+} (6 cultures at each concentration). After ~10 min the frequency of ciliary beat had increased to a new stable level which, in the case of the Hanks' solution containing 10^{-3} M Ca^{2+} , was approximately similar to the initial control. At this time the dose-effect relationship of PGE_2 (Upjohn) on the frequency of ciliary beat was investigated in each group of cultures by increasing the level of the prostaglandin in the Rose chambers while maintaining constant the correspondent calcium concentration (Fig. 2).

In a different set of experiments three cultures were decalcified in Ca-free Hanks' with 2 mM EGTA but in the presence of 10^{-8} M PGE_2 (Fig. 1). Finally in three other cultures PGE_2 was introduced into the Rose chamber after ciliary activity had been arrested by decalcification in Ca-free 2 mM EGTA Hanks' solution.

As observed in our preliminary investigations⁷, the frequency of ciliary beat in mammalian ciliated cells can be reversibly modified by changing the extracellular Ca^{2+} concentration. However, the present experiments indicate that the stimulation of oviductal cilia by PGE_2 is fairly independent of calcium concentration in the medium. Note that in Fig. 2, especially at PGE_2 concentrations higher than 10^{-7} M, the rate of ciliary beat increases to a maximum and demonstrates no statistical difference over a rather wide range of Ca^{2+} concentrations.

The infusion of Ca-free Hanks' solution containing 2 mM EGTA in the Rose culture chamber produces a consistent

biphasic time-dependent decrease of the frequency of ciliary beat (open circles in Fig. 1). However, when this same experiment is performed in the presence of 10^{-7} M PGE_2 , the frequency of cilia beat shows a transient increase followed by an exponential decrease that results in the arrest of ciliary activity within approximately 40 min (solid circles in Fig. 1). This effect mimics that produced by the Ca ionophore, A23187, in cultures of ciliated cells of the rabbit trachea and oviduct⁷.

When PGE_2 at 10^{-7} M concentration is introduced into the culture chamber after ciliary activity has been arrested by 2 mM EGTA in Ca-free Hanks', a typical rapid increase followed by a rapid decrease in the frequency of ciliary beat is observed. Further increasing the PGE_2 concentration to 10^{-6} M produces transiently an additional smaller increase in frequency. However, this response could not be elicited again by 10^{-5} M PGE_2 .

Calcium has been implicated in excitation-contraction coupling⁸ and in coupling the avoiding response of ciliary reversal in protozoa⁹ as well as coupling non-motile processes such as stimulus-release of neurotransmitter and endocrine hormones¹⁰. The present experiments suggest that calcium may also be involved in the stimulus-response coupling of hormonal stimulus to ciliary motions produced by PGE_2 . It has been proposed that much of the pharmacology of prostaglandins can be understood on the basis of their facilitation of Ca^{2+} movement across biological membranes¹¹. Our data appear to corroborate this idea, suggesting that the stimulation produced by PGE_2 must be associated with the release of intracellular calcium as: (1) the increase in the frequency of ciliary beat produced by PGE_2 is fairly independent of calcium levels in the medium (Fig. 2); (2) although the complex time decay in the frequency of ciliary beat produced by decalcification is not sufficient to support the idea of multi-compartmentalisation of calcium in ciliated cells, the transient increase, followed by an exponential decrease, in ciliary activity observed in the presence of PGE_2 (Fig. 1) is consistent with a prostaglandin-stimulated release of calcium from intracellular compartments; and (3) the transient activation produced by PGE_2 in ciliated cells maintained in Ca-free medium and arrested by calcium-depletion indicate that in these cells there must be a pool of intracellular

calcium which can be released by PGE_2 and which may, transiently, stimulate ciliary activity before diffusing to the extracellular space.

The similarity of the effect of PGE_2 to that of the ionophore A23187 (Fig. 1) is not surprising as it has been suggested that prostaglandins may act as a 'calcium ionophore' in subcellular structures¹².

The data presented here are the first indication of calcium-dependent coupling of hormonal stimulus to ciliary activity. They suggest that PGE_2 stimulates ciliary motion by releasing intracellular bound or sequestered calcium, a mechanism that has also been suggested to explain the effect of prostaglandins in other tissues¹².

Prostaglandins have been found in the wall of the oviduct¹³ where ciliary actions have been shown to be directly implicated in ovum transport¹⁴. The present work, therefore, may serve as a model to gain knowledge of the mechanism of action of hormonal influences on ciliary activity and also to understand further the role of prostaglandins in oviductal function.

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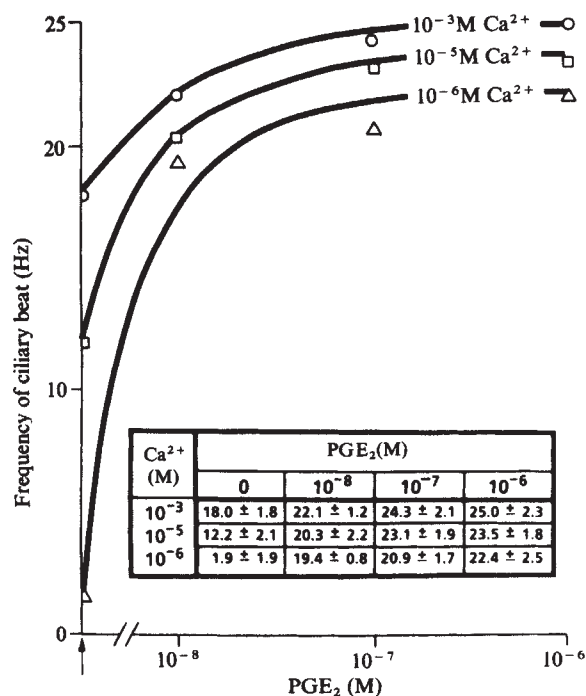


Fig. 2 Effect of various concentrations of PGE_2 on the frequency of ciliary beat in cultures of ciliated cells from the rabbit oviduct equilibrated in Hanks' solution containing various concentrations of calcium ions. Each point is the average $\pm$ s.d. of ~ 120 samples of frequency of ciliary beat.

An ATP-dependent Ca^{2+} -pumping system in dog heart sarcolemma

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Since the pioneering studies of Ringer of almost a century ago^{1,2}, it has been known that the contraction of heart cells requires the presence of calcium in the extracellular medium. Ca^{2+} penetrates into the sarcoplasm where it either directly activates troponin C (ref. 3), or indirectly conveys the contraction signal to the myofibrils, possibly by inducing Ca^{2+} release from intracellular stores (sarcoplasmic reticulum, SR)⁴⁻⁶. After contraction Ca^{2+} must obviously leave the heart cell again to prevent its progressive accumulation. As the electrochemical potential of Ca^{2+} across the sarcolemma would prevent its passive outflow, the efflux of Ca^{2+} is an energy requiring process. Most of the Ca^{2+} is apparently ejected from heart cells in exchange for Na^+ (refs 7, 8), on an electrophoretic antiporter which exchanges three Na^+ ions for one Ca^{2+} (ref. 9). However, there is some evidence¹⁰⁻¹⁴ of a specific ATPase in cardiac sarcolemma, which could also have a role in the energy-dependent ejection of Ca^{2+} . One important difficulty in trying to establish the existence of a specific Ca^{2+} -ATPase in cardiac sarcolemma lies in the fact that most of the preparations used in such studies have been heavily contaminated by other subcellular fractions (mitochondria, SR) which also possess ATP-dependent Ca^{2+} transporting systems. In the present work we describe an ATP-dependent Ca^{2+} transport system which can be conclusively ascribed to heart sarcolemma. We show that a heart vesicular preparation particularly enriched in sarcolemmal markers accumulates Ca^{2+} in the presence of ATP. That the process takes place in sarcolemmal vesicles is demonstrated by the fact that the accumulated Ca^{2+} is promptly and completely released in exchange for Na^+ .

Sarcolemmal vesicles from dog heart were prepared using a method slightly modified from that of Jones *et al.*¹⁵. The final pellet was washed twice and resuspended in 160 mM KCl, 20 mM HEPES pH 7.4 (K-medium), or, when required, in 160 mM NaCl, 20 mM HEPES pH 7.4 (Na-medium). Experiments on $\text{Na}^+/\text{Ca}^{2+}$ exchange were carried out as described by Reeves and Sutko⁸. The $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ was measured after unmasking the total activity with Na-SDS¹⁵. The Ca^{2+} -ATPase was measured as described in ref. 15, in the presence of 20 μg oligomycin per mg protein. Each experiment described has been performed at least four times, with at least three different sarcolemmal preparations. The Na^+ content of the suspensions was determined by atomic absorption spectroscopy, and the protein content using a modification of the method of Lowry *et al.*¹⁶.

The preparation used in the present work is very highly enriched in sarcolemmal vesicles, with specific activities of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ varying between 80 and 105 μmol of phosphate liberated per mg of protein per h. The preparation accumulates Ca^{2+} at the expense of a transmembrane Na^+ gradient⁸, 80% of the uptake (60 nmol per mg protein) taking place within the first 10 s. The sarcolemmal vesicles, however, also hydrolyse ATP in the presence of both Ca^{2+} and Mg^{2+} , with a specific activity of 8.5 μmol per mg protein per h. Concomitant with the hydrolysis of ATP, the vesicles accumulate Ca^{2+} , to a maximal level, in the experimental conditions used, of 46 nmol per mg of protein in 10 min (Fig. 1). The apparent K_m of this Ca^{2+} transport system lies between 0.2 and 0.6 μM .

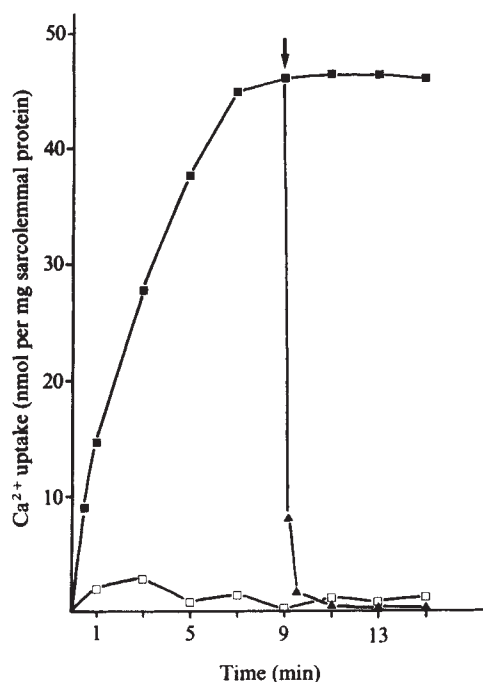


Fig. 1 ATP and Mg^{2+} -dependent Ca^{2+} uptake in dog heart sarcolemma. Sarcolemmal vesicles (20 μg of protein suspended in K-medium) were preincubated at 37°C in 2 ml of a medium containing 160 mM KCl, 20 mM HEPES, 1 μg oligomycin, 5 mM MgCl_2 , 1 mM ouabain pH 7.4. After 5 min, 150 nmol $^{45}\text{CaCl}_2$ were added: 2 min later, the first aliquot was withdrawn, and immediately after (time zero) 1 mM K_2ATP (final concentration) was added. Further aliquots (100 μl) were withdrawn at the times indicated, immediately filtered through Millipore filters (pore diameter 0.22 μm) and washed with 1 ml of ice-cold K-medium, containing 1 mM LaCl_3 , to avoid Ca^{2+} movements during the 15-s filtration time. The values are corrected for the Ca^{2+} found associated to the sarcolemma before the addition of ATP (average, 28 nmol per mg of protein). The experiments were carried out at 37°C. In none of the experiments was more than 20% of the total ATP added consumed. ■, No supplementary additions; ▲, at the arrow the ionophore A23187 (final concentration 5 μM) was added; □, no ATP or no Mg^{2+} .

Since two other membrane systems in heart—the mitochondria and SR—accumulate Ca^{2+} in the presence of ATP, appropriate controls were carried out. The possibility of the involvement of the mitochondrial system can be excluded, since oligomycin (20 μg per mg protein, an amount in great excess of that necessary to inhibit completely the ATP-dependent Ca^{2+} transport in mitochondria) and the uncoupler FCCP (1 μM) do not affect Ca^{2+} accumulation in sarcolemmal vesicles. Oxalate at 0.1 to 5 mM also had no effect, making the contribution of SR unlikely. Figure 1 also shows that Ca^{2+} is indeed accumulated into the inner vesicular space, and not superficially bound. The Ca^{2+} -specific ionophore A23187 discharges the accumulated Ca^{2+} completely and very rapidly. Also of interest is the observation that the accumulation of Ca^{2+} requires the presence of Mg^{2+} in the medium.

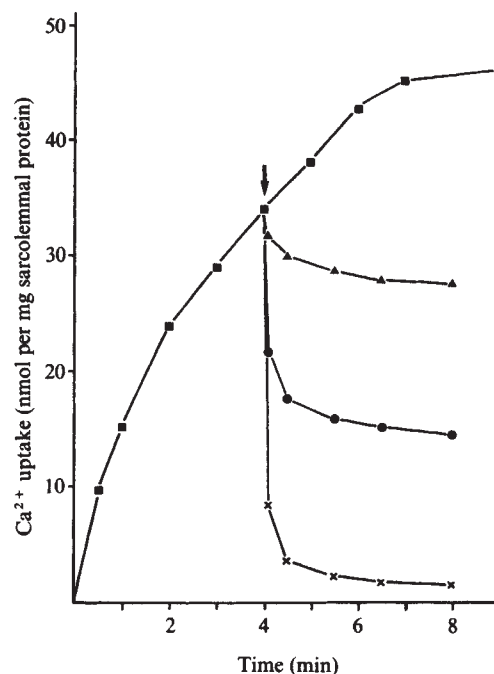


Fig. 2 Na^+ -induced release of the Ca^{2+} accumulated by dog sarcolemma in the presence of ATP. Experimental conditions as in Fig. 1, except for the addition (at the arrow) of NaCl (or KCl) to give the final concentrations indicated below. ■, No additions, or 30 mM KCl, or 5 mM NaCl; ▲, 10 mM NaCl; ●, 20 mM NaCl; ×, 30 mM NaCl.

The sarcolemmal preparation used in the present experiments was derived from an oxalate-treated microsomal fraction. The possibility therefore existed that some oxalate could still be associated with SR vesicles contaminating the sarcolemmal preparation. Thus the ATP-dependent uptake of Ca^{2+} could be due, totally or in part, to the SR vesicles: the experiment shown in Fig. 2 rules out this possibility. The SR does not possess a $\text{Na}^+/\text{Ca}^{2+}$ exchange system. However, the addition of Na^+ to the medium, after the vesicles have taken up about 35 nmol of Ca^{2+} per mg of protein, rapidly discharges the accumulated Ca^{2+} . The discharge is only partial at 10 mM added Na^+ , but complete at 30 mM. Ionic strength and osmolarity effects are ruled out by the lack of effect of 30 mM KCl, demonstrating that the Ca^{2+} releasing effect of Na^+ is specific. It is clear, then, that the system under study is constituted by (sarcolemmal) vesicles into which an ATP and Mg^{2+} -dependent system pumps Ca^{2+} , and from which Na^+ specifically releases it.

One more possibility had to be ruled out before concluding that the sarcolemmal vesicles possess a Ca^{2+} transporting system that depends directly on the hydrolysis of ATP. Since it is to be expected that a considerable fraction of the vesicles used in this study have an inside/out polarity, the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ could

in principle have preloaded the sarcolemmal vesicles with Na^+ , which could then be released in exchange for Ca^{2+} (ref. 9). Addition of large amounts of Na^+ could then release the accumulated Ca^{2+} again. Measurements of the Na^+ concentration in the outside medium, however, have indicated values below $20 \mu\text{M}$, at which no $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ activity could be detected. In addition, the inclusion of ouabain in the reaction medium (followed by preincubation for 1 h at 15°C to allow equilibration of the inhibitor across the vesicle membrane) had no effect on the observed movements of Ca^{2+} in the experimental conditions used. Finally, the inclusion in the medium of Na^+ concentrations increasing from $100 \mu\text{M}$ to 1 mM (lower than those necessary to observe any release of the accumulated Ca^{2+} , but already adequate to sustain a certain level of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ activity¹⁷), failed to increase the uptake of Ca^{2+} . An increase in Ca^{2+} uptake, on the other hand, would have been expected if the accumulation of Ca^{2+} were due to the discharge of a Na^+ gradient previously established by the operation of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$. We can therefore conclude that the sarcolemma of heart cells possesses, in parallel with the $\text{Na}^+/\text{Ca}^{2+}$ exchange system, an ATP-dependent system which specifically transports Ca^{2+} . This system seems to have a higher affinity for Ca^{2+} than does the $\text{Na}^+/\text{Ca}^{2+}$ exchange system, but a lower transport rate (Fig. 2). These differences could be of importance in considering the respective roles of the two systems in heart physiology.

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Evidence for two populations of excitatory receptors for noradrenaline on arteriolar smooth muscle

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We have recorded the responses of arteriolar smooth muscle cells to iontophoretically applied noradrenaline. Records of both muscle movement and muscle membrane potential were made. We found that two distinct types of response could be detected, depending on the position of the noradrenaline micropipette. One type of response consisted of a localised constriction near the noradrenaline source: this effect could be abolished by the α -antagonist phentolamine and was not associated with a change in arteriolar membrane potential. The other type of response was a depolarisation similar to the excitatory junction potentials (e.j.ps) produced by sympathetic nerve stimulation. These observations suggest that there are two populations of receptors for noradrenaline on arterioles, and could explain the paradoxical failure of α -antagonists to block neuromuscular transmission at some autonomic end organs such as the vas deferens, arteries and arterioles.

Intracellular recordings were made from very short segments of arteriole (~ 0.25 electrical length constants; for details see refs 1, 2). Both the membrane potential and diameter of the arteriolar segment were monitored, the latter by use of an inverted compound microscope¹ and a video tape recorder. Muscle movements were measured after the end of the experiment by playing back the video signal frame by frame. The use of such short lengths ensured that any membrane potential change occurring in the preparation would be detected by the intracellular recording electrode. This point was checked by impaling the preparation with two independent microelectrodes, one at each end of the segment. Invariably if both recording electrodes detected an e.j.p. following nerve stimulation, passage of current through one electrode caused a change in membrane potential which was detected by the other electrode. With these very short preparations the electrotonic potentials could not be distinguished from those which would result from current injection into a simple resistance/capacitance circuit².

The normal response of such preparations to low frequency supramaximal nerve stimulation (0.4 Hz) was the initiation of e.j.ps with mean amplitudes in the range 1.8–12 mV. These potentials did not produce a detectable movement although occasionally an e.j.p. reached threshold and initiated a muscle action potential resulting in constriction of the whole segment. E.j.ps were not reduced in amplitude by either phentolamine mesylate, tolazoline hydrochloride, prazosin hydrochloride or labetalol hydrochloride (each at $1 \times 10^{-6} \text{ g ml}^{-1}$) even at stimulation frequencies as low as 0.1 Hz.

Attempts were made to mimic nerve stimulation by iontophoretic application of noradrenaline from a second microelectrode (noradrenaline 0.5 M, electrode resistance 150–500 M Ω). The most common response to application of a brief pulse of noradrenaline (5–10 ms, 50 nA) was a localised constriction of the arteriole in the region near the iontophoretic pipette (Fig. 1). These responses were not associated with a change in membrane potential. There was no obvious relationship between the separation of the recording electrode and the iontophoretic electrode and the ability to produce localised constriction. In several experiments constriction of the arteriole occurred within a few micrometres of the recording electrode but even this was not accompanied by a change in membrane potential. The responses not associated with a change in membrane potential (Fig. 1) could be abolished by the addition of phentolamine mesylate ($1 \times 10^{-6} \text{ g ml}^{-1}$) to the bathing solution.

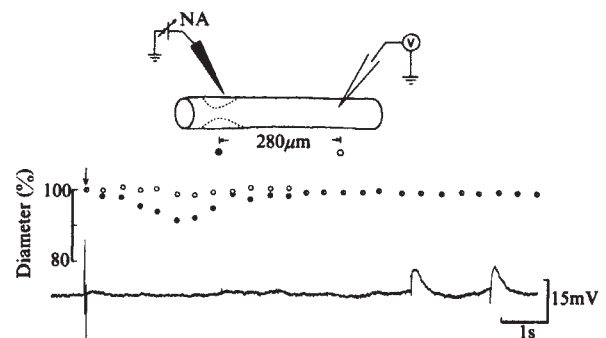


Fig. 1 Effect of iontophoretic application of noradrenaline (NA) on the diameter of an isolated arteriolar segment (upper trace, circles) and on its membrane potential (lower trace). The arteriole had a diameter of $63 \mu\text{m}$ and a length of $350 \mu\text{m}$; after the application of noradrenaline (pulse duration, 10 ms; current, 50 nA at arrow) the arteriole constricted but the constriction was limited to the area near the iontophoretic pipette (●, see experimental sketch). No change in diameter was detected at the position of the recording electrode (○) which was placed some $280 \mu\text{m}$ further along the arteriolar segment. This response was not associated with a detectable change in membrane potential. At the end of the lower trace, two e.j.ps produced by perivascular nerve stimulation can be seen. These were not associated with a detectable change in arteriolar diameter.



Fig. 2 Effect of iontophoretic application of noradrenaline at a 'reactive area'. A pulse of noradrenaline (duration 10 ms, current 50 nA) produced a depolarisation which initiated an action potential (lower trace), and a generalised constriction of the entire arteriolar segment (upper trace).

On some occasions areas on the arteriolar surface were found where iontophoretically applied noradrenaline produced a depolarisation (Fig. 2). The position of the iontophoretic electrode was critical; in any one experiment some 10–20 trials would be required before an area where noradrenaline caused a depolarisation was found. When this depolarisation initiated an action potential, the entire arteriolar segment constricted (Fig. 2). The delay between the start of the iontophoretic pulse and the onset of depolarisation was highly variable, ranging from <10 ms to 500 ms. Part of this delay is probably due to the difficulty of expelling catecholamines from high resistance microelectrodes because the delay could be shortened by using short high-frequency trains of iontophoretic pulses. Depolarising responses to iontophoretically applied noradrenaline could be detected in the presence of phentolamine (1×10^{-5} g ml $^{-1}$) but we have not yet been able to rule out the possibility that phentolamine had reduced the amplitude of the depolarisation. During a prolonged intense depolarising response (30–40 mV change in membrane potential) to noradrenaline produced by long iontophoretic pulses (100 ms) e.j.ps could not be detected. This suggests that neuronally released transmitter and applied noradrenaline may initiate conductance changes with similar reversal potentials.

There is an abundance of data to indicate that noradrenaline is synthesised, stored, and released by many sympathetic post-ganglionic nerve terminals³. However, in smooth muscle tissues which receive an excitatory noradrenergic innervation such as arteries, arterioles and vasa deferentia, it has been difficult to explain why responses to exogenously applied noradrenaline differ in some ways from those to nerve stimulation. For example, stimulation of sympathetic nerves innervating the vas deferens produces a two-component contraction, only one component of which is abolished by traditional α -receptor blocking drugs⁴. The contractile response produced by exogenous noradrenaline can be readily abolished⁵. Noradrenaline causes a contraction of arteries in concentrations which do not produce a change in membrane potential⁶; but e.j.ps not associated with movement, are detected from arterioles and arteries in response to sympathetic nerve stimulation^{7,8}. For reasons such as these the role of noradrenaline as the primary transmitter has been questioned^{9,10}.

We suggest that the difficulty in duplicating responses produced by neuronal stimuli by the application of exogenous noradrenaline arises because the smooth muscle cells have two distinct populations of excitatory receptors for noradrenaline. One group of the receptors which cause membrane depolarisation are the same receptors (junctional receptors) as those which are activated by neuronally released noradrenaline. The other (extrajunctional) receptors have the pharmacological properties of traditional α -receptors. When they are activated, they cause constriction without an associated membrane potential change. In arterioles either the neuronally released transmitter does not have access to extrajunctional receptors or if it does it reaches them in such a low concentration that no contractile responses

can be detected (this may not be the case for more densely innervated tissues such as the vas deferens).

These observations are consistent with the view that noradrenaline is a primary transmitter in the sympathetic nervous system. However, responses to sympathetic nerve activity can only be mimicked if noradrenaline is applied to certain restricted areas; the receptors activated at these areas are different from conventional α -receptors which therefore seem to play no part in neuromuscular transmission.

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Proprioceptors with central cell bodies in insects

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A common feature of invertebrate sense organs is that the cell bodies of the sensory neurones are located in the periphery, close to the site of stimulus reception. For some phyla of invertebrates examples are known where sensory cells have their somata inside the central nervous system (CNS), but none are yet known for the insects. In this report we describe a proprioceptive organ in the locust thorax that consists of an elastic strand that is supplied with terminal branches of a small group of sensory neurones. The somata of these neurones are not located in the periphery, but inside the third thoracic ganglion. Our findings indicate that at least a small number of cell bodies in insect ganglia may belong to sensory neurones, rather than to motoneurones, interneurones or neurosecretory cells.

In locusts the hindlegs are involved in a number of behavioural tasks like stepping, stridulating, kicking, swimming, steering in flight, grooming and help in changing body position. These movements do not follow all-or-nothing programmes imposed by the CNS but are modified in amplitude and duration in response to sensory information especially that from the proprioceptors of the leg itself. Although the proximal joints between thorax, coxa and trochanter of the hindlegs have a major role in whole leg movements, more is known about the more distal joints between femur and tibia and tibia and tarsus^{1,2}. We looked for additional internal proprioceptors in the proximal leg joints of the migratory locust (*Locusta migratoria*) by infusing cobaltous solutions distally^{3,4} into nerves 2 and 3 of the metathoracic segment. One of the terminal peripheral branches of nerve 3 splits into a meshwork of arborisations on a connective tissue strand between the thorax and the trochantin (Fig. 1) without supplying any detectable somata. This is in contrast to nearby chordotonal organs (for example, cxtnCO in Fig. 1). Backfills of these terminal arborisations on the strand show 6–8 neurone somata, 10–15 μ m in diameter, in the anterior lateral region of the metathoracic ganglion (Fig. 2). These neurones show rather restricted fields of arborisation compared to locust motoneurones⁵.

It seemed possible that this new structure was a degenerating larval muscle, but electron microscopic sections of the strand and terminal nerve arborisations did not show any similarity

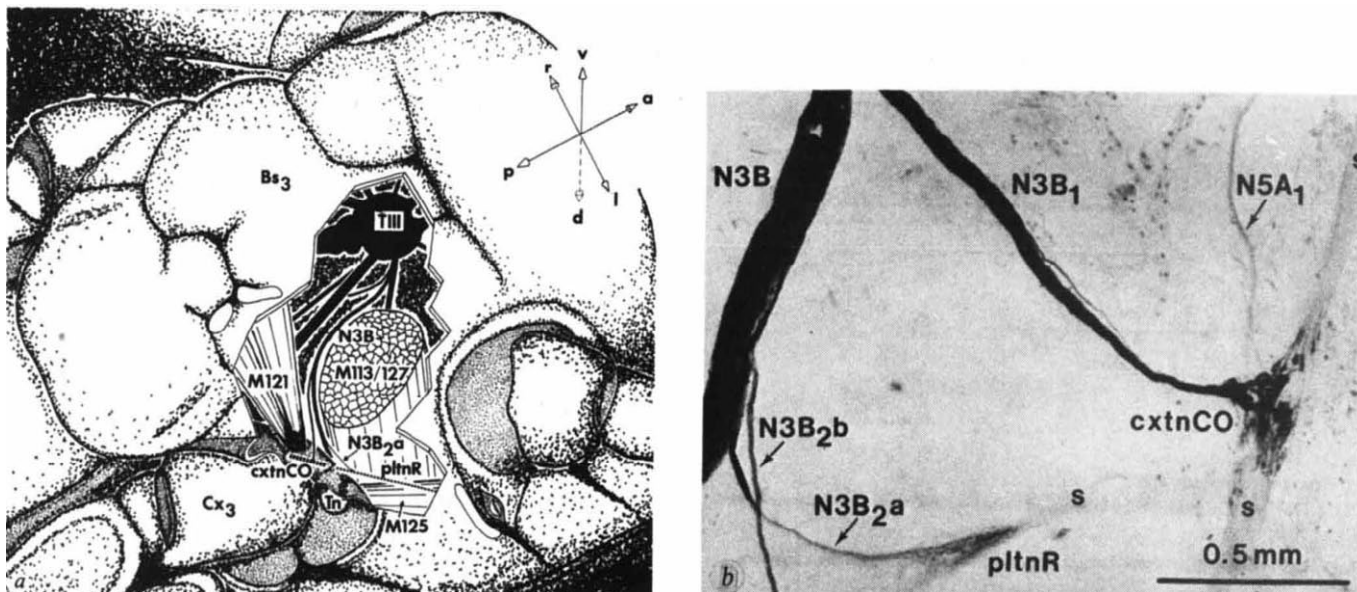


Fig. 1 Morphological features of the pleurotrochantal receptor (pltnR) in the metathorax. *a*, Ventro-lateral view of the third thoracic segment (metathorax) of the migratory locust. A large window has been cut into the sternal cuticle (Bs_3) and the joint membrane (narrow stipling) of the hindleg coxa (Cx_3). Air sacs and trachea have been removed exposing some muscles (M121, M113/127, and M125) and the metathoracic ganglion (TIII) with all anterior nerve roots except for nerve 2. Two sense organs of the leg joint (cxtnCO, tnplR) are innervated by small branches which emerge from the middle tract of the third nerve root (N3B). a, Anterior; p, posterior; r, right; l, left; v, ventral; d, dorsal; Tn, trochantin. *b*, Peripheral cobalt stain showing branches of nerve 3B. Note the prominent cell bodies in the chordotonal organ (cxtnCO) in contrast to the receptor (pltnR); s, connective tissue strand. The relative position of the two sense organs does not correspond with the natural one in this microscopic wholemount.

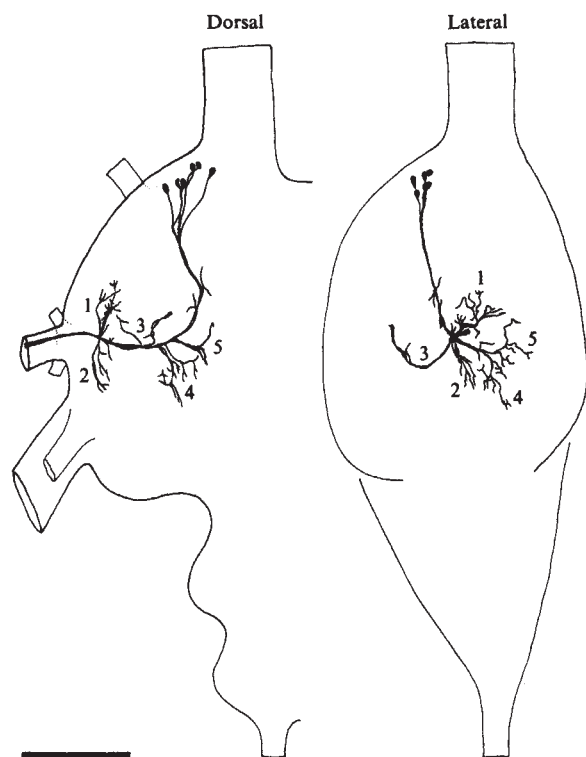


Fig. 2 Cobalt filled and silver intensified central projections in the metathoracic ganglion and somata of six neurones supplying the receptor. Camera lucida drawing of the hemiganglion, viewed dorsally and laterally. Same dendritic areas are indicated with corresponding numbers. Scale bar, 0.2 mm.

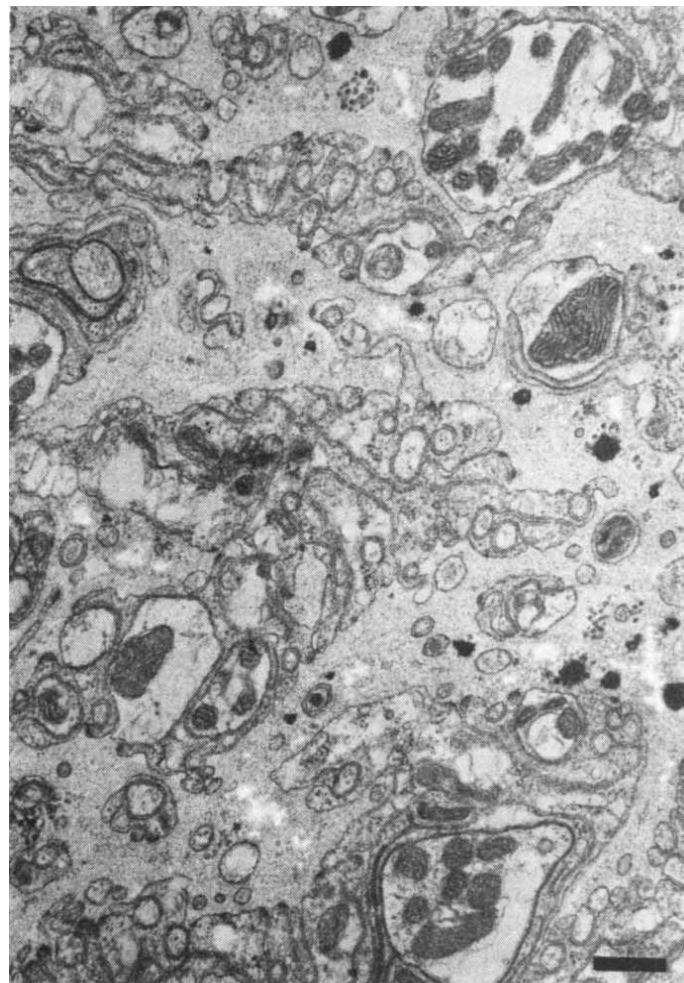


Fig. 3 (right) Electron microscopic cross-section of the receptor strand containing terminal branches of nerve 3B_{2a}. Characteristic features: large (for example upper right) nerve profiles, usually surrounded by glia and containing many mitochondria, and small nerve profiles, wrapped in glia or embedded in extracellular material without glial sheath (middle). Scale bar, 0.5 μ m.

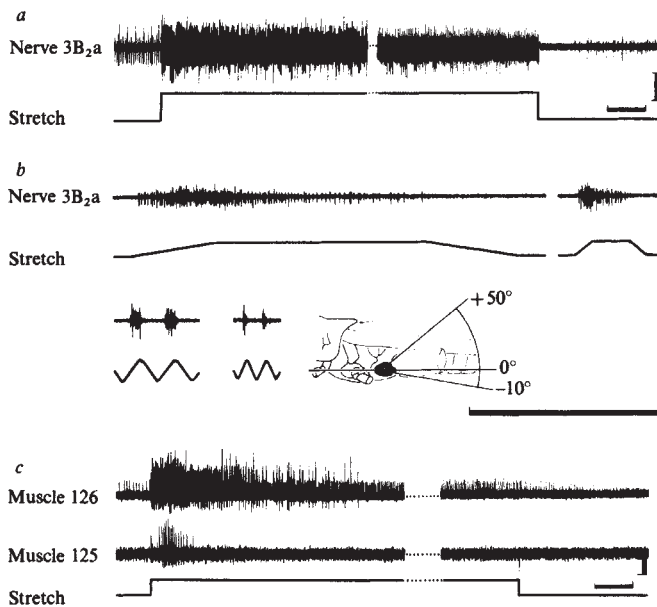


Fig. 4 Afferent and efferent responses to mechanical stimulation of the receptor. *a*, Phasic-tonic afferents (upper trace) after stretching (lower trace) the strand record from distal end of cut nerve 3B2a. Note the pronounced off-response. *b*, The effect (upper trace) of slow and fast trapezoid movements (lower traces) applied to the coxa from -10° to $+50^{\circ}$ as indicated. Range corresponds to normal range in behaviour. *c*, Phasic-tonic reflex response (upper trace) in the motoneurone supplying muscles 125 and 126 and phasic response in a myogram of muscle 125 (middle trace) to stimulation of the receptor as in 3*a* (lower trace). Calibrations: horizontal, 1 s; vertical, 0.5 mm. Dotted lines are omitted parts on continuous recordings.

with muscle tissue (Fig. 3). The strand can be found in all larval instars and it persists in the adult without change.

Further evidence for a sensory function was obtained by recording from the distal part of the terminal nerve branch which supplies only the strand. Afferent volleys of spikes from the organ indicated the presence of several units responding in a phasic-tonic manner to trapezoid stretching of the strand (Fig. 4*a*). This identifies it as a stretch receptor. The attachments of the strand suggested a proprioceptive function characteristic of the strand receptor, and recording afferent spikes when moving the whole coxa shows a response to low and high angular velocities (Fig. 4*b*). This information may be used by the CNS for position control in still animals and for monitoring angular velocities of the moving coxa, as stretching the receptor strand causes increased motoneurone discharge of the coxal depressor muscles (Fig. 4*c*). Contractions of these muscles counteract forces that elongate the strand.

The metathoracic receptor does not seem to be the only one of this new morphological type in locusts. Peripheral cobalt stainings showed similar structures in a comparable position in the fore- and middle leg and others spanning the coxa-trochanter joints of all three leg pairs.

All previously described insect mechanoreceptors have their sensory cell bodies in the periphery as most invertebrate sense organs do. Exceptions have been found in some annelid⁶ and crustacean⁷⁻⁹ species, for which sensory neurones with somata in the CNS have been reported. The discovery of this new type of proprioceptor in the locust brings the insects in line with these other invertebrate groups. The location of the sensory structure in the proximal leg joints and their appearance at the light-microscopic level reminds one of certain strand receptors reported for decapod Crustacea⁷. However, there is a striking difference in signal propagation: locust organs send their information to the CNS by means of action potentials, whereas crustacean receptors only transmit graded potentials^{8,9}.

The ontogeny of the described sensory cells remains an unsolved problem: do they develop like insect mechanoreceptor cells from late ectodermal cells during embryogenesis with cell bodies migrating into the CNS or are they derived from neuroblasts like motoneurons and interneurons?

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Dopamine depolarisation of mammalian primary afferent neurones

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Dopamine (DA) is an important neurotransmitter or neuromodulator in the mammalian nervous system. As such, it is implicated in the aetiology and therapy of various disease conditions—for example, Parkinson's disease, schizophrenia, Huntington's disease and tardive dyskinesia. However, only limited electrophysiological information is presently available concerning dopamine receptors in the mammalian nervous system¹, and there are only three reports²⁻⁴ in which intracellular techniques have successfully recorded the action of DA on individual central neurones. In all cases, DA depolarised the respective neurones. In the periphery, DA is reported to hyperpolarise superior cervical ganglia⁵⁻⁹. However, this hyperpolarisation has been shown to be due to activation of α -adrenoreceptors and not to a response of DA on a DA receptor^{8,9}. Peripheral DA actions have also been described presynaptically^{10,11}, but are difficult to study electrophysiologically for technical reasons. As a result, little is known at the membrane level about the effects of drugs thought to modulate or interact with DA receptors. In the present report, we describe a depolarising action for DA on the cat dorsal root ganglion.

Lower lumbar dorsal root ganglia (DRG) were dissected from adult cats (2-5 kg) that were anaesthetised intraperitoneally (i.p.) with a mixture of α -chloralose (60 mg kg⁻¹) and pentobarbital (5 mg kg⁻¹). The isolated ganglia were maintained at 37 °C in physiological solution aerated with 95% O₂ and 5% CO₂. A physiological solution of the following composition was used to superfuse the preparation (in mM): NaCl, 117; KCl, 4.7; CaCl₂, 2.5; MgCl₂, 1.2; NaH₂PO₄, 1.2; NaHCO₃, 25 and glucose, 11.5. Standard intracellular recording techniques¹² using K-citrate (2 M) filled microelectrodes were used. Known concentrations of drugs were added to the superfusate.

We have been investigating the pharmacology of a γ -aminobutyric acid (GABA) receptor that has been characterised on the cell body of cat dorsal root ganglia¹². As a result of *in vitro*¹²⁻¹⁴ or *in vivo*¹⁵ techniques with ganglia from adult animals, it has been assumed that receptors on the somata of primary afferent neurones may be similar to those on their terminals. There have been two reports^{16,17} which suggest that neuroleptic drugs (agents thought to interact with central DA receptors) may also affect GABA-GABA receptor interactions.

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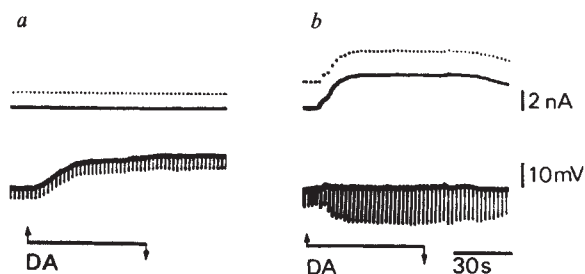


Fig. 1 Effects of bath applied DA (5×10^{-4} M) on membrane potential and resistance. *a*, Upper pair of tracings (current monitor) represents constant hyperpolarising current pulses. Lower tracing (voltage monitor), solid line represents membrane potential from which anelectrotonic responses deflect downward. *b*, Same as *a* except a current is applied manually during the DA application to prevent the drug-induced depolarisation. In these conditions, the membrane resistance increases during DA.

To examine this possibility, we applied the putative DA-antagonists chlorpromazine (CPZ) and haloperidol while monitoring iontophoretically induced GABA depolarisations. Neither CPZ nor haloperidol at concentrations to 10^{-5} M altered the GABA response. However, at 10^{-5} M, haloperidol occasionally depolarised the membrane and would block an action potential induced by cathodal stimulation through the recording electrode. A similar depolarising action for haloperidol had been reported at a DA receptor on a vertebrate neuronal cell hybrid¹⁸. As a result of this depolarising action by this DA antagonist, we undertook the following experiments to investigate whether DA has a direct action on these cells.

After impaling a neurone its effective membrane resistance ($1/\text{conductance}$) was determined from the ratio of voltage/current measurements. Electrotonic potentials required for the above measurements were generated by brief repetitive anodal current pulses delivered through the recording electrode. When DA was applied to the preparation, a sustained membrane depolarisation with little conductance change was recorded (Fig. 1*a*). Because DA depolarised the membrane at a slow rate, the membrane potential could be held constant during the DA application by passing a continuous hyperpolarising current through the recording electrode. In these 'manual clamp' conditions¹⁹, DA decreased conductance (Fig. 1*b*). In one other report³, the DA depolarisation was usually not associated with a conductance change. The lack of a conductance change during the unclamped dopamine response is probably due to the membrane rectifying properties of the dorsal root neurones. Because of the rectifying properties we intend to determine the

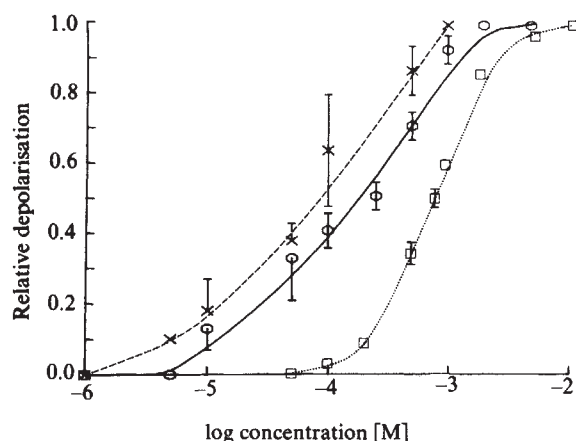


Fig. 2 log concentration-response relationships for DA ($K_a = 1.3 \times 10^{-4}$ M), apomorphine ($K_a = 7 \times 10^{-5}$ M) and GABA ($K_a = 7.8 \times 10^{-4}$ M). $\times$, Apomorphine; $\circ$, dopamine; $\square$, GABA.

ionic mechanism of this DA-induced depolarisation using voltage clamp techniques. Preliminary results show that unlike the GABA-induced depolarisation chloride is not involved in the DA-induced depolarisation, whereas a decreased potassium conductance is, at least, contributory.

Dopamine, at various concentrations, produced similar slow membrane depolarisations in 75% of the cells to which it was applied (209/275). In no instance did DA hyperpolarise the membrane. The complete concentration-response relationships for DA from 13 neurones are depicted in Fig. 2. For comparison, various concentrations of apomorphine ($n = 4$), a specific DA agonist, and GABA ($n = 10$) were also applied to the preparation. Apomorphine ($ED_{50} = 7 \times 10^{-5}$ M) was at least twice as effective as DA ($ED_{50} = 1.3 \times 10^{-4}$ M) in causing a membrane depolarisation. The maximum depolarisation produced by DA and apomorphine was about 10 mV. Both DA and apomorphine produced sustained, non-desensitising depolarisations for

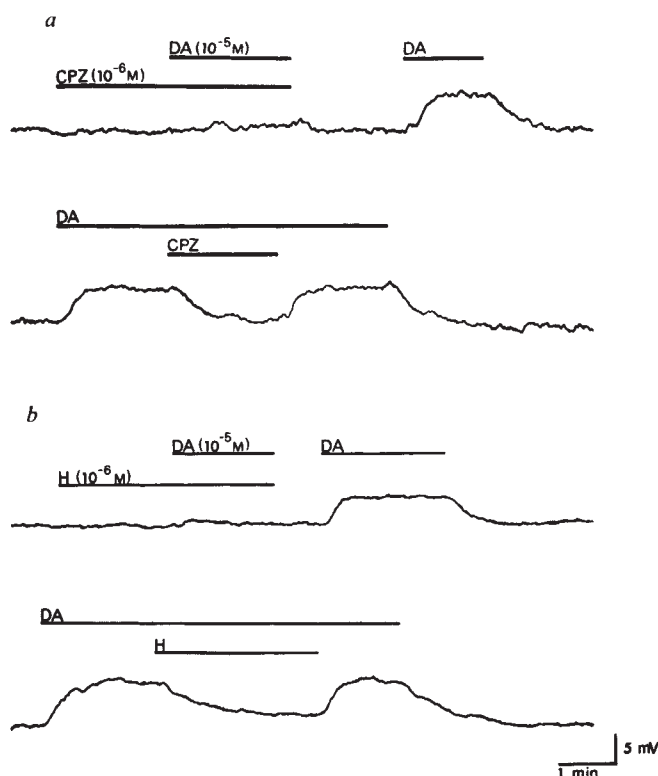


Fig. 3 Effects of antagonists on dopamine depolarisation. *a*, Chlorpromazine (CPZ) effects on membrane potential and on DA-induced depolarisation. *b*, Haloperidol (H) on membrane potential and on DA-induced depolarisation.

as long as the drug was in contact with the preparation. This action differed from one observed when GABA is applied for a prolonged period because the GABA depolarisation rapidly declines as the receptor becomes desensitised^{12,13}.

Of the drugs tested, only chlorpromazine (CPZ) and haloperidol antagonised the action of DA (Fig. 3). CPZ (10^{-6} M) had no effect on the resting membrane potential but antagonised the depolarising action of DA (Fig. 3*a*). In the continued presence of DA, the addition of CPZ inhibited a DA depolarisation. Haloperidol (10^{-6} M) produced a similar antagonistic action (Fig. 3*b*).

Other amines were applied to the sensory ganglion by the superfusion technique. Although noradrenaline depolarised the ganglion, it did not depolarise to the same degree as DA, even at concentrations 10 times greater than that of DA. The depolarisation induced by noradrenaline could not be antagonised by phentolamine, an α -adrenoreceptor antagonist. Propanolol, a β -adrenoreceptor antagonist was also ineffective in blocking the action of the amines. Furthermore, adrenaline

and serotonin were less effective depolarising agents than noradrenaline. This weak effect of serotonin was unexpected as it has been shown to be active at the nodose ganglion²⁰ and the superior cervical ganglion²¹. Isoprenaline did not depolarise.

The concentrations of DA ($ED_{50} = 1.3 \times 10^{-4}$ M) required to depolarise the ganglion are high. Nonetheless, we believe that this action is specific because the responses are antagonised by classical DA antagonists and not affected by the α - or β -adrenoreceptor antagonists. In a recent study with isolated toad spinal cords, catecholamines, including DA, at concentrations similar to those used here, depolarised dorsal root terminals²². In addition, there is evidence that dopaminergic neurones are present in the mammalian spinal cord²³. These authors have described a differential distribution for DA within the cord; DA concentration was found to be two to three times higher in the dorsal horn than in the ventral horn. This distribution pattern would support the fact that DA receptors on the sensory ganglion soma may also be present on their terminals. At present, there is no published evidence regarding DA binding to dorsal root ganglia.

The dorsal root ganglion may provide us with essential information about the interaction of DA with its receptor and of drugs thought to alter its action at the membrane level. With this knowledge a better insight into the therapeutic approach to various diseases of the central nervous system may be achieved.

Serotonergic neurones are not involved in action of L-dopa in Parkinson's disease

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Under normal circumstances, L-dopa is converted to dopamine (DA) in DA-containing neurones of the corpus striatum by the enzyme aromatic L-amino acid decarboxylase¹. Although L-dopa has been used widely to treat Parkinson's disease, the site of its decarboxylation to DA in the parkinsonian striatum is unknown. It was initially assumed that dopa administration enhances DA synthesis and release in surviving nigrostriatal neurones²⁻⁶. Subsequent *in vitro* studies provided evidence that exogenous dopa might also be taken up, decarboxylated⁷⁻⁹, and released as DA^{8,10} by serotonergic terminals, suggesting that non-dopaminergic neurones also may mediate some of dopa's therapeutic effects¹⁰. During long-term dopa treatment in parkinsonism, its efficacy often declines¹¹, and diurnal fluctuations in its effectiveness (the 'on-off' phenomenon) can become manifest in previously responsive patients¹². If the effects of dopa depend solely on surviving dopaminergic neurones, its decreasing efficacy with time could imply continuing degeneration of striatal terminals^{11,13}. However, if exogenous dopa can also affect striatal DA transmission by being decarboxylated in serotonergic neurones, the declining therapeutic efficacy could reflect involvement of serotonergic neurones in the pathology of Parkinson's disease; this has previously been reported, but remains controversial¹⁴⁻¹⁷. Therefore, we examined the decarboxylation of exogenous dopa in an animal model of parkinsonism—rats with unilateral nigrostriatal lesions¹⁸—which were further subjected to lesions of the striatal serotonergic projections. Our data indicate that combined destruction of striatal dopaminergic and serotonergic terminals has little additional effect, beyond destroying dopaminergic neurones, on dopa's ability to enhance striatal DA release and do not favour the concept that serotonergic neurones are involved in mediating the therapeutic effects of L-dopa.

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Groups of 5–10 Sprague–Dawley rats (200–250 g; Charles River) had free access to water and Big Red rat chow (Charles River). Lesions in midbrain dorsal and median raphe nuclei were produced electrolytically (2 mA, 20 s in the dorsal and 2 mA, 25 s in the median raphe nucleus). One month later, unilateral nigrostriatal lesions were performed in raphe-lesioned and control rats by injecting stereotactically 8 μ g 6-hydroxydopamine (OHDA) in 4 μ l saline containing 0.2 mg ml⁻¹ ascorbic acid into the right anteromedial substantia nigra (level A 2400, according to König and Klippel¹⁹). This was immediately followed by an additional injection of 4 μ g in 2 μ l into the right medial forebrain bundle (level A 4000) to increase the lesion's severity. Two weeks after the OHDA treatments, animals were injected with L-dopa (100 mg per kg, intraperitoneally), placed in metal cylinders (35 cm diameter), and their turnings (full circles) were counted during the 5-min interval between 40 and 45 min after the injection. Rats were then decapitated and both striata were dissected separately and frozen on dry ice. Tissues were homogenised in ice-cold distilled water; buffered aliquots were assayed for tyrosine hydroxylase (TH)²⁰ and dopa decarboxylase (DDC) activities²¹, and deproteinised aliquots for 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), using high-performance liquid chromatography with electrochemical detection²². Serotonin in small striatal samples was determined by an enzymatic-isotopic method²³.

Since striatal DA concentrations after dopa administration can no longer indicate the extents of nigrostriatal lesions, we used TH activity for this purpose. TH activities and DA levels were highly correlated ($r = 0.91$) in OHDA-lesioned striata of rats not receiving dopa (F.H. *et al.*, in preparation). After OHDA injections, there were marked reductions (by 90–95% compared to control or contralateral striata) in TH activity in lesioned striata, indicating severe unilateral destruction of the nigrostriatal system in all groups of animals (Table 1). The raphe lesions produced marked reductions (by approximately 90%) in serotonin levels in both OHDA-lesioned and contralateral striata (0.75 ± 0.15 and 0.55 ± 0.13 ng per mg protein, respectively; mean $\pm$ s.e.m.), compared with levels in control striata (6.68 ± 1.18 ng per mg protein). Although dopa administration has been reported to decrease brain serotonin levels²⁴, we found no decrease in another group of unlesioned, dopa-treated animals (6.82 ± 0.52 ng per mg protein, $n = 10$). DDC activity was reduced in OHDA-lesioned striata by 76–87% compared with

those in control and contralateral unlesioned striatal tissues. Despite severe destruction of the nigrostriatal system, DDC activity was relatively less reduced than TH activity, confirming that striatal DDC exists both within and outside dopaminergic neurones²⁵. DDC activity in striata of animals with unilateral nigrostriatal lesions was not decreased further if animals were subjected to raphe lesions. Dopa administration caused no significant changes in TH and DDC activities in lesioned or control striata.

Nigrostriatal lesions produced marked ipsilateral depletions of striatal DOPAC and HVA (Table 1). Dopa administration increased the concentrations of these DA metabolites in unlesioned, contralateral striata. It also markedly elevated DOPAC and HVA levels in lesioned striata, indicating that decarboxylation of exogenous dopa occurs despite a marked reduction in the number of DA-containing nerve endings. In rats with combined raphe-nigral lesions, the increases in striatal DOPAC and HVA after dopa administration were similar to those observed in animals with unilateral nigrostriatal lesions alone. This indicates that dopa decarboxylation in striata containing a diminished number of dopaminergic terminals is not altered after additional destruction of the striatal serotonin input.

Dopa reportedly increases locomotor activity in rats, and this action is further amplified after destruction of central catecholaminergic neurones by intraventricular OHDA²⁶. If brain serotonergic neurones were also destroyed, dopa's effects on motor activity were either reduced²⁷ or unchanged²⁸. In rats with unilateral nigrostriatal lesions, dopa administration induces contraversive circling behaviour, attributed to enhanced DA synthesis and release within the lesioned striatum¹⁸. In the present study, dopa-treated rats with combined raphe-nigral lesions displayed vigorous turning away from the lesioned side; this behaviour did not differ from circling noted in rats with unilateral nigrostriatal lesions alone (Table 1). This finding contradicts a recent report²⁹ showing diminished rotational response to L-dopa when both striatal serotonergic and dopaminergic neurones have been destroyed unilaterally. In this report, the unilateral lesioning of serotonergic neurones (by injecting 5,7-dihydroxytryptamine into one lateral ventricle), probably caused an additional asymmetry between the two striata, which by itself could have affected turning behaviour^{30,31}. In our study, raphe lesions reduced serotonin levels by about the same extent in both striata; such lesions do not affect the circling associated with asymmetries in the nigrostriatal system^{32,33}.

To examine the relative contribution of DDC within serotonergic neurones to total striatal DDC activity, rats were

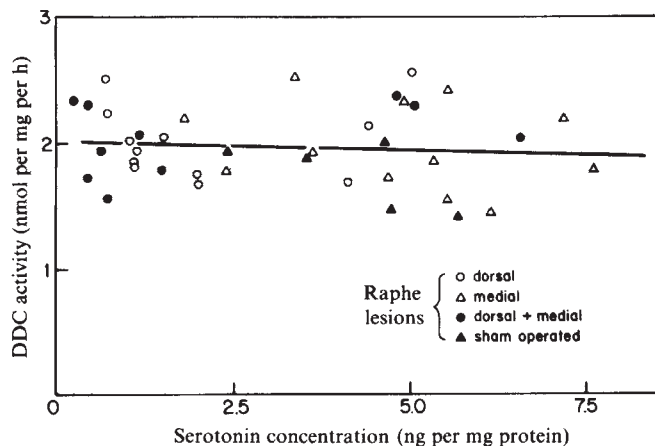


Fig. 1 Striatal dopa decarboxylase activity and serotonin concentrations in rats with electrolytic lesions in the midbrain dorsal or median raphe nuclei, or in both nuclei. Sham-operated rats were used as controls. Rats were killed 2 weeks after the lesions were produced, and the right and left striata from each rat were pooled and analysed for dopa decarboxylase activity (using [$1\text{-}^{14}\text{C}$]dopa as substrate)²¹ and for serotonin²³. Regression line was calculated by the least squares method; $r = -0.10$.

subjected to electrolytic lesions in dorsal or median raphe nuclei, or in both nuclei. Dorsal and combined dorsal-median raphe lesions reduced striatal serotonin content (Fig. 1), confirming the presence of a projection from the dorsal raphe to the striatum in the rat^{34,35}. Striatal DDC activity (measured using [$1\text{-}^{14}\text{C}$]dopa as substrate) did not decrease despite marked reductions in serotonin. This finding is consistent with our previous observation (Table 1) that a raphe lesion does not further reduce striatal DDC activity in rats with nigrostriatal lesions. These data suggest that the decarboxylase contained within serotonergic neurones contributes insignificantly to the total DDC activity of the striatum.

Our study shows that in rats with severely damaged nigrostriatal systems, an additional lesion destroying striatal serotonergic neurones does not alter the ability of exogenous dopa to enhance DA release or to cause a behaviour (contraversive circling) thought to be induced by DA release. Hence, in our experimental conditions, dopa's effects possibly are not significantly dependent on its uptake in, decarboxylation in, or

Table 1 Effect of dopa on striatal DOPAC and HVA concentrations in rats with combined raphe and unilateral nigrostriatal lesions

	TH (nmol per mg per h)	DDC (nmol per mg per h)	DOPAC (ng per mg)	HVA (ng per mg)	Turning (turns per 5 min)*
Controls	0.153 ± 0.010	2.89 ± 0.23	0.58 ± 0.10	0.64 ± 0.06	0
OHDA lesion:					
OHDA-lesioned	0.016 ± 0.008†	0.42 ± 0.05†	0.02 ± 0.02†	0.04 ± 0.01†	
Contralateral	0.148 ± 0.007	2.24 ± 0.21	0.43 ± 0.06	0.43 ± 0.04	
OHDA lesion + DOPA					
OHDA-lesioned	0.008 ± 0.004†	0.49 ± 0.12†	1.77 ± 0.25‡	1.40 ± 0.20‡	20.5 ± 6.6
Contralateral	0.154 ± 0.017	2.11 ± 0.21	2.65 ± 0.53§	2.05 ± 0.39§	
OHDA + raphe lesions + DOPA					
OHDA-lesioned	0.007 ± 0.001†	0.56 ± 0.09†	1.34 ± 0.76‡	1.40 ± 0.62‡	31.0 ± 7.0
Contralateral	0.128 ± 0.009	2.95 ± 0.73	3.65 ± 1.55§	2.37 ± 0.84§	

Values are means ± s.e.m. Rats were lesioned electrolytically in the dorsal and median raphe nuclei. One month later, these animals and other control rats were injected unilaterally with 8 µg OHDA into the substantia nigra and 4 µg into the medial forebrain bundle. Two weeks after the OHDA lesions were produced, animals received 100 mg per kg L-dopa intraperitoneally and were put in metal cylinders for measurement of turning behaviour between 40 and 45 min after injection. Rats were then decapitated and the enzyme activities (TH and DDC) and DOPAC and HVA concentrations were measured in both striata. Data were subjected to analysis of variance followed by Scheffe's test.

* Full circling away from the OHDA-lesioned side.

† Different from controls and from contralateral side by $P < 0.01$.

‡ Different from lesioned striatum of untreated rats by $P < 0.01$.

§ Different from controls and from contralateral side of lesioned untreated rats by $P < 0.05$.

release as DA from striatal serotonergic terminals⁸⁻¹⁰. Thus our findings do not suggest a major role for serotonergic neurones in mediating dopa's effects in Parkinson's disease. In intact striata, DDC activity is localised predominantly in DA-containing terminals (Table 1). The DDC activity remaining after nigrostriatal lesions could be contained within surviving DA-containing neurones and in other striatal constituents, such as the capillaries^{36,37}. DA formed from dopa by DDC within capillary endothelium probably lacks functional significance, since it may not be able to traverse the blood-brain barrier and reach striatal synapses^{36,38,39}. The rotational behaviour⁴⁰ and striatal DA formation (E.M. *et al.*, in preparation) after dopa administration are not abolished when rats with unilateral nigrostriatal lesions are given a drug that blocks vascular DDC, indicating that, in lesioned rats, dopa is also converted to DA outside striatal capillaries. Further studies should determine if this decarboxylation occurs exclusively within surviving dopaminergic neurones or also in other striatal compartments.

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Black widow spider toxin-induced calcium fluxes and transmitter release in a neurosecretory cell line

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Several polypeptide neurotoxins affect presynaptic functions by interfering with chemical neurotransmission. This group of toxins includes botulinum toxin¹, tetanus toxin², β -bungarotoxin³ and black widow spider toxin (BWSTx)⁴⁻⁶. While the effect of the first three toxins is mainly a rapid and severe block of neurotransmitter release, BWSTx affects transmission by a massive stimulation of mediator release⁴⁻⁶. Despite various hypotheses put forward to explain the action of BWSTx at the level of nerve terminals, there is still a considerable degree of uncertainty as to the cation dependence of venom action⁷⁻¹¹. Study of the toxin mode of action at the biochemical level has been hampered by the complexity and cellular heterogeneity of the preparations used, neuromuscular junction¹⁰ or synaptosomes⁶. PC12 cell line, derived from a rat pheochromocytoma¹², seems to be an excellent model in view of its property of synthesising and storing noradrenaline, dopamine and acetylcholine, and releasing them in depolarising conditions¹³. We have recently shown that highly purified BWSTx stimulates secretion from PC12 cells of previously taken up radioactive dopamine (DA) and noradrenaline (NA) (ref. 14 and manuscript in preparation). We report here that the earliest detectable event after toxin treatment of such cells is a massive increase of cytosolic calcium.

When PC12 cells grown in monolayer and loaded with ³H-labelled DA are exposed to BWSTx in a nominally Ca²⁺-free medium, containing 1 mM EGTA, the radioactivity released in the medium equals that of untreated controls (less than 1% of total intracellular radioactivity per min). Extensive washing of

the cells and substitution of the latter medium with a Ca²⁺-containing medium (1.8 mM) results in a release of more than 30% of total labelled catecholamines in the following 5 min (Fig. 1). The amount of transmitter release is quantitatively comparable with that obtained with an equal amount of toxin added to a Ca²⁺-containing medium (dashed line, Fig. 1). BWSTx-induced release of ³H-DA is completely blocked if cells are pre-exposed to micromolar concentrations of concanavalin A or if toxin is challenged with monospecific antibodies before addition to cultures. However, if lectin or antibodies are added after toxin is bound to cell membrane in EGTA, Ca²⁺-triggered effect (see Fig. 1) is maintained (data not shown). Furthermore Ca²⁺-dependent release of DA after toxin treatment is temperature-dependent, being reduced by over 80% when temperature is lowered from 37° to 8°C.

Table 1 Effect of several agents on Ca²⁺ flux and dopamine release induced by BWSTx and K⁺

Condition	Percentage inhibition of maximum effect			
	Ca ⁴⁵ flux		DA release	
	BWSTx	K ⁺	BWSTx	K ⁺
TTX (1 × 10 ⁻⁶ M)	0	0	0	0
Sea anemone Tx II (5 × 10 ⁻⁶ M)	0	0	0	0
Co ²⁺ (1 × 10 ⁻³ M)	0	80	0	75
(2 × 10 ⁻³ M)	35	98	30	95
Cd ²⁺ (2 × 10 ⁻³ M)	ND	ND	0	90
Verapamil (5 × 10 ⁻⁵ M)	32	70	10	75
Concanavalin A (5 × 10 ⁻⁷ M)	100	0	100	0

Experimental conditions were as described in Fig. 2, except that BWSTx (8 nM) and K⁺ (56 mM) were directly added to cultures in standard HR medium (1.8 mM CaCl₂). ⁴⁵Ca uptake and ³H-DA release were measured after 6 min incubation at 25°C. Each value represents the mean of duplicate assays in a typical experiment. Values are expressed as a per cent of maximum effect in control treated cultures. ND, not determined.

It thus seems that BWSTx binds to PC12 membrane in the absence of Ca^{2+} but is unable to induce catecholamine secretion unless physiological concentrations of divalent cations are added to the medium. The next question which arises concerns the site at which external calcium ions are necessary to make the toxin effect apparent. Is the toxin directly activated by Ca^{2+} ions or does it induce transmitter release by allowing Ca^{2+} ions to enter the cells? To test the latter question we made use of PC12 cells exposed to toxin in a medium containing EGTA. After 10 min unbound toxin was extensively washed out and the cells quickly exposed to a medium containing 1.8 mM ^{45}Ca . As shown in Fig. 2a, immediately after the addition of the Ca^{2+} -Ringer a massive influx of ^{45}Ca is measurable in cells pretreated with a saturating concentration of toxin (7 nM). This influx is several times higher than that induced by depolarising concentrations of K^{+} or by 0.7 nM toxin. In the latter case net stimulation of ^{45}Ca uptake tends to level off after about 100–150 s. Release of ^3H -DA was followed in conditions identical to those used for the Ca^{2+} flux experiments (Fig. 2b). Stimulation of DA release over background is very rapid and its time course seems to be concomitant with that of ^{45}Ca influx at both toxin concentrations. Interestingly, although the uptake of ^{45}Ca elicited by 7 nM toxin is 10- to 12-fold higher than that due to 0.7 nM toxin (or 56 mM K^{+}), the ratio, as far as DA secretion is concerned, is only 2.5–3.0. This suggests that only a proportion of the Ca^{2+} entering cells is saturating those intracellular sites involved in secretion, the rest being perhaps responsible for the cellular swelling observed as a consequence of long-term exposure to high toxin concentrations (unpublished results). Note that while

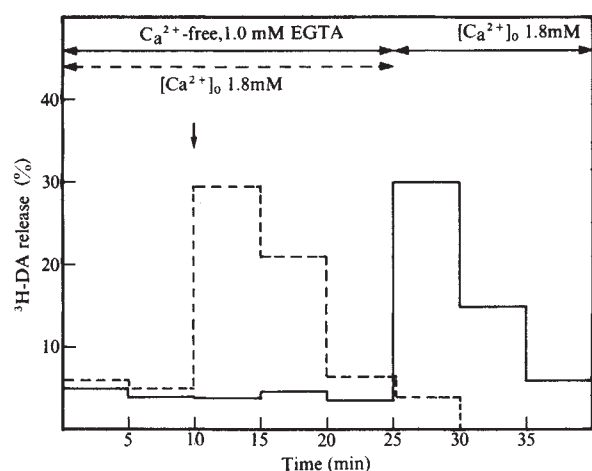


Fig. 1 Influence of Ca^{2+} on dopamine release in response to BWSTx. PC12 cells were grown as monolayer on collagen-coated Petri dishes (35 mm) at a density of 10^6 cells per dish. Cells were washed free of culture medium and incubated at 37°C for 60 min in 1 ml of HEPES-Ringer (ref. 13) (HR) supplemented with $1 \mu\text{Ci}$ per dish ^3H -DA, 10^{-4} M pargyline and $0.5 \text{ mg BSA ml}^{-1}$. Specific activity of ^3H -DA (Amersham) was $6\text{--}7 \text{ Ci mmol}^{-1}$. At the end of the uptake period, cultures were brought to 25°C , washed three times with HR (containing 1.8 mM CaCl_2). Sister cultures were washed with a modified Ca^{2+} -free HR buffer (also containing 1 mM EGTA). Cultures were then incubated for 5-min periods with 1-ml aliquots of the respective buffers. The releasing medium was collected from the dishes by careful suction directly into scintillation vials. After two 5-min intervals, experiment was started (arrow) by addition of 5 pmol of purified BWSTx (molecular weight 125,000)⁶. Toxin was present only in the following 5-min interval. Dashed line refers to cultures in the presence of HR. Continuous line refers to cultures in EGTA-HR. In this set, Ca^{2+} -containing HR was replaced after three EGTA-HR washes following toxin addition. At the end of the experiment cells were solubilised with 2% SDS. Radioactivity was counted in 5 volumes of Aquasol-2. ^3H -DA release is expressed as a per cent of total radioactivity present in the cells at the beginning of the experiment. The cells remain firmly attached to the substrate during the whole experiment.

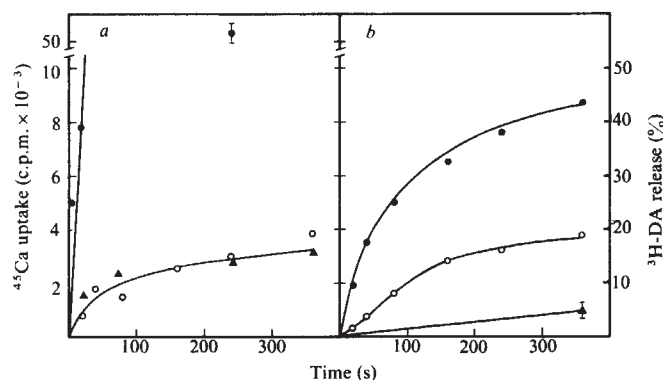


Fig. 2 Time course of ^{45}Ca uptake and ^3H -DA release induced by two different toxin concentrations. *a*, PC12 cells were grown in conditions identical to those described in Fig. 1. Cultures, after washing with EGTA-HR, were incubated for 10 min at 25°C with the same buffer but containing, when appropriate, 0.7 (○) and 7.0 (●) nM BWSTx and bovine serum albumin 0.5 mg ml^{-1} . The medium containing toxin was then removed and cultures challenged with 1 ml of HR containing 1.8 mM ^{45}Ca ($5 \times 10^6 \text{ c.p.m. ml}^{-1}$) for the indicated time intervals. 56 mM KCl was present in K^{+} -stimulation experiments (▲). Uptake was terminated by washing the dishes quickly three times with HR and then solubilising the cells in 2% SDS. Background Ca^{2+} uptake ($0.05\text{--}0.1 \text{ nmol per min per } 10^6 \text{ cells}$) was subtracted from total counts to obtain the values plotted above. Possible exchange of Ca^{2+} during the course of our measurements was ignored. *b*, PC12 cells preloaded at 37°C with ^3H -DA as described in Fig. 1, were equilibrated at 25°C in EGTA-HR also containing the same toxin concentrations as in (*a*) for 10 min. After removal of toxin release was started by addition of standard HR. Background release of tritiated DA (less than 1% per min) is given (▲). DA release expressed as in Fig. 1. Each point represents the mean from three different experiments. Net Ca^{2+} fluxes of comparable magnitude were observed also if BWSTx was added in a standard HR medium. However kinetics were somewhat modified because of toxin diffusion and binding to cells. BWSTx did not cause uptake of ^{45}Ca by non-secretory cells such as normal or transformed chick fibroblasts and rat myoblasts.

these results suggest initial Ca^{2+} entrance as a primary event in the induced release, a secondary mobilisation of internal stores of Ca^{2+} is not to be excluded.

Two possible mechanisms by which BWSTx might enable Ca^{2+} to enter the cells are: (1) activation of physiologically operating voltage-dependent Ca^{2+} channels, which have been recently described by Stallcup in PC12 cells¹⁵; or (2) induction of new channels somewhat analogous to those formed by toxin on artificial lipid bilayers¹⁶. We found that in agreement with previous findings, both transition metals and verapamil (a calcium-channel blocker) fully inhibit K^{+} -induced Ca^{2+} influx as well as DA release^{17,18} (Table 1). Such full inhibition was not observed when the same compounds were tested on toxin, thus suggesting that the major part of BWSTx effect does not involve direct activation of voltage-dependent Ca^{2+} channels. The moderate reduction in Ca^{2+} entry (and hence in DA release) observed at high Co^{2+} concentrations might be due to Co^{2+} permeating in place of ^{45}Ca , but being unable to stimulate release (see ref. 18). An alternative explanation might involve BWSTx acting through two distinct mechanisms—induction of 'specific' channels and a secondary contribution from Co^{2+} and verapamil-sensitive channels, perhaps activated by a concomitant depolarisation. However, support for the idea that Ca^{2+} entry elicited by toxin is unlikely to involve voltage-dependent channels comes from the observation that, at variance with potassium¹⁸, transmitter release induced by the toxin is not inhibited by high Ca^{2+} concentrations (10 mM) (data not shown). Finally, ^{45}Ca flux induced by BWSTx does not require the presence of Na^{+} , being enhanced by a factor of two, possibly

through a mechanism discussed earlier¹⁵, in a Na^+ -free-sucrose medium; even dopamine release is not appreciably affected in the same experimental conditions.

The main result of our experiments is the demonstration that BWSTx produces a rapid and massive influx of Ca^{2+} ions in a toxin-responsive, neurosecretory cell. The main consequence of Ca^{2+} influx is the release of neurotransmitters presumably through a typical exocytotic process^{19,20}. Although we have no direct evidence that other cations besides Ca^{2+} cross the cell membrane as a consequence of toxin action, in our conditions neither Na^+ nor Mg^{2+} alone seem to effectively substitute for Ca^{2+} in inducing transmitter release. The present work also confirms and extends the previous observations^{21,22} on the antagonistic effect of concanavalin A (see Table 1) by showing that the lectin inhibits BWSTx-induced Ca^{2+} uptake. On the basis of the available evidence, it seems reasonable to postulate that binding to a concanavalin A-sensitive 'receptor' is an obligatory step for the formation of the previously discussed Ca^{2+} 'channels'.

We thank Dr A. Levi for comments and suggestions and Dr L. Greene for providing the PC12 cell line. This work was in part supported by a CNR grant under the Controllo Crescita Neoplastica programme. This paper is dedicated to Mr B. Capparella (deceased February 1979), who by his unique skill in spider collection, has helped past and present research on Latrodectism.

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X-ray-induced *in vitro* neoplastic transformation of human diploid cells

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Techniques have recently been developed to identify and score quantitatively neoplastic transformation caused by X rays in cultured cells derived from rodents¹. Because of their relevance to estimates of human cancer risk, it is clearly desirable to carry out similar experiments with cultured cells of human origin. The present report describes for the first time the neoplastic transformation *in vitro* of human diploid cells by X-ray irradiation into cells which can progress *in vitro* into advanced stages of neoplastic development, namely, to form colonies in agar and give rise to tumours when injected into nude mice.

We used an early passage of skin fibroblast strain KD cells, originally derived from a skin biopsy of an adult woman^{2,3}. The cultures were in their fifth cell passage, dating from the original biopsy. Chromosome G banding analysis carried out on the cells on arrival indicated a normal diploid human karyotype. The doubling time of the KD cells as determined by growth curve analysis⁴ was 30-32 h.

The cells were routinely maintained in Eagle's minimum essential medium (MEM) containing 10% fetal bovine serum and 1% human serum (GIBCO) and incubated at 37°C. A dose-response curve for cell survival was carried out as described elsewhere⁵ (see Fig. 1). The scheme for transformation experiments is described in detail in Fig. 2 legend. All cells were fed twice a week. Four separate experiments have been carried out using antipain⁶ or β -oestradiol. In each experiment control cultures included unirradiated, untreated cells as well as cells unirradiated, but treated with antipain or β -oestradiol. These were set up in equal numbers to the experimental flasks and subcultured in the same manner.

Within 2 months of irradiation, discrete foci were detected in the pretreated, irradiated cultures which had been left untouched following the 1:12 split and were now at high density. The foci, consisting of cells with a subtle irregularity in cell to cell orientation, were visible under phase microscopy. Two or three foci were initially detected in each experiment in both antipain- and oestradiol-pretreated, irradiated cultures, suggesting that these compounds were equally effective in potentiating radiation-induced transformation. Within 3 months, 30-40 of these foci per experiment were observed in most of the high-density flasks but not in those which had been given a further subculture (Fig. 2). The latter exhibited these foci after transferring the cells at high density (2×10^6) to 100-mm Petri dishes.

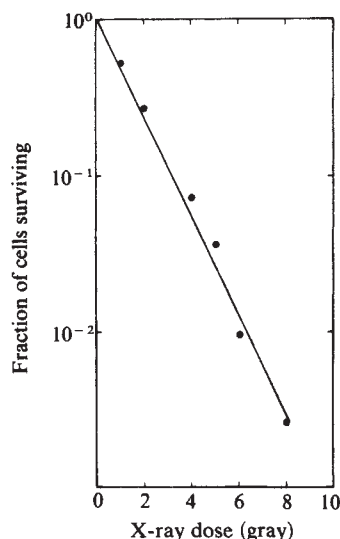


Fig. 1 Cell survival of KD cells following irradiation by X rays, from a Sieman's 300 kVp constant potential generator with an added filter of 0.2 mm copper.

The foci increased in size. A multilayering of cells made them clearly visible to the naked eye (Fig. 3). No foci appeared in the unirradiated cells or in cells treated with antipain or oestradiol alone.

By exchanging the medium for medium containing low calcium concentrations⁷, the cells in the morphologically transformed foci underwent an additional, more dramatic morphological alteration (Fig. 3), whereas the normal cells degenerated within 24 h.

Chromosomal G banding analysis carried out on cells isolated from the primary foci showed a human chromosomal constitution with a diploid to near-diploid number of chromosomes

(range 46–49). A more detailed karyotype will be published elsewhere. Saturation density determined on the transformed cells cultured from the foci showed a twofold increase in saturation density over that of the normal. The transformed cells but not the normal parental cells were agglutinable by $25 \mu\text{g ml}^{-1}$ of concanavalin A (Miles-Yeda).

The ability to proliferate at low serum concentrations (1%) was not confined to the transformed human cells⁸ as in rodent systems^{1,4}, but was also a property of the normal KD cells grown in the same conditions (10^5 cells per 60-mm Petri dish).

Cells isolated from the multilayered foci were seeded in semi-solid agar⁹, where they proliferated to form distinct spherical colonies (Fig. 3) with a cloning efficiency of 2–3%. None of the unirradiated KD cells or the irradiated but untransformed cells (seven samples of each) was able to proliferate in agar. Colonies isolated from agar could be recultured in liquid medium or further propagated in agar.

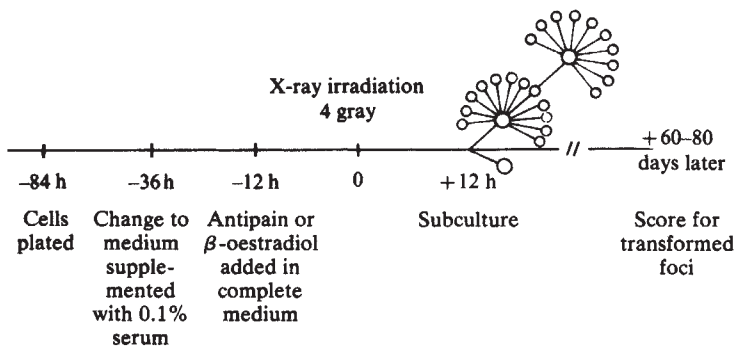


Fig. 2 Experimental design for the induction of human cell transformation *in vitro* by 4 gray of X rays (1 gray = 100 rad). Cells were trypsinised (0.25% trypsin), suspended in complete medium and plated in tissue culture flasks (75 cm²) (Falcon) at 2.6×10^5 cells. Two days later, the medium was replaced with MEM containing 0.1% serum for 24 h, whereby cell proliferation was greatly reduced. After 24 h, medium was again exchanged, this time for complete medium (11% serum) containing either $6 \mu\text{g ml}^{-1}$ of the protease inhibitor antipain⁶ or $1 \mu\text{g ml}^{-1}$ β -oestradiol (Sigma). Experimental cultures were irradiated 12 h later with 4 gray of X rays as previously described⁵. All cultures were divided into two 10–12 h after irradiation and reseeded in fresh complete MEM without antipain or oestradiol. At near confluency, one of the two flasks was subcultured into 12 flasks, the other being left undisturbed. In each experiment, when these 12 flasks reached confluency, one flask was subcultured 1:10 and thus defined as 'continuously passaged', and the remaining 11 flasks were left at high density.

The ultimate proof of the neoplastic nature of the cells transformed *in vitro* is their ability to form tumours in an appropriate host. Swiss *nu/nu* mice were injected intradermally with 5×10^6 cells (suspended in 1 ml medium) into the subscapular region of animals X-ray-irradiated 24 h earlier with 450 rad or unirradiated¹⁰.

Whereas the five transformed lines tested formed colonies in agar, three gave rise within 6 weeks both in the irradiated and unirradiated mice to tumours which have been characterised as noninvasive fibrosarcomas. Cells cultured from the tumours possessed a human karyotype which will be described in detail elsewhere. None of the unirradiated or the irradiated but untransformed cells (seven samples of each) gave rise to tumours in the nude mice.

The present experiments show that human diploid cells can be transformed *in vitro* into tumorigenic cells by X-ray-irradiation. Cultures which were treated and irradiated in the non-dividing state, as well as those which were allowed only four or five cell divisions before reaching confluency (and not subcultured further), did not exhibit transformed foci. This indicates that cell

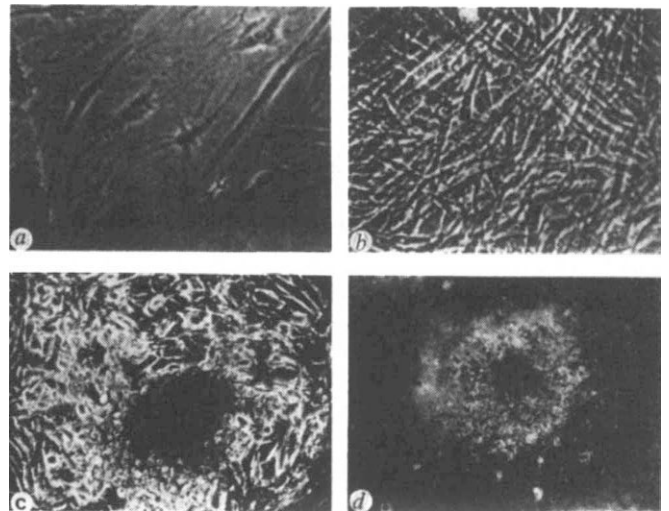


Fig. 3 a, Human KD cells in irradiated but untransformed cultures. Phase $\times 58$. b, Human KD cells transformed *in vitro* by 4 gray. Note the criss-cross pattern of the cells. Phase $\times 58$. c, The same area as in b 24 h after incubation in low calcium. Phase $\times 58$. d, X-ray-transformed KD cells growing in 0.33% agar. $\times 12$.

replication is required for the fixation and expression of radiation-induced neoplastic transformation in diploid human cells, and a quantitative assessment is now in progress. This requirement has been shown to exist in the case of rodent cells transformed *in vitro* by radiation^{11–13} or chemicals^{14,15} and in human cells transformed *in vitro* by chemicals³. It was of interest that cells which were not recognisable as transformed at low density expressed transformation when replated at high density in close contact.

The complex experimental design used here takes advantage of the following. (1) The cells which were well adapted to complete medium were subjected to conditions of serum deprivation for a period of 24 h, thus reaching a complete quiescence, indicated in these conditions by specific conformational changes in chromatin (Hoen, personal communication). (2) The addition of complete medium 24 h later led to a synchronous wave of DNA synthesis and treatment of the cells at that point enabled the capturing of cells entering S phase.

We do not know the mechanism of action of oestradiol and antipain in this system. Neither compound exhibited mitogenic activity at the concentration used and both seemed equally effective. We have previously shown that antipain, a protease inhibitor, when present in cultures during irradiation enhanced the yield of X-ray-induced neoplastic transformation in hamster and mouse cells¹⁶. Oestradiol has been used to potentiate chemically induced transformation¹⁷ and although steroids have many functions, they do have some protease inhibitor activity¹⁸. Thus, one could speculate that the enhancing action of both compounds may be in part through a common mechanism related to this property.

Although the above experimental regime has been successful for the induction and detection of radiation-induced transformation in the KD cells, it must be stressed that we are relatively ignorant of the best experimental design for inducing transformation and detecting transformants in human cells. Note, for example, that although partial cell synchrony and potentiation of transformation by hormones was a necessary step in the transformation of some diploid human cells by chemical carcinogens¹⁷, it was not required for the induction of transformation in other diploid cells³. Because the latter³ were, in fact, the KD cells, we are now evaluating other experimental designs to induce and detect transformation by radiation in the human cells. The ability to transform human cells *in vitro* by

X-ray irradiation opens up a wide range of studies which should aid in the assessment of human risk to radiation-induced carcinogenesis and in the evaluation of treatments to inhibit its expression and progression.

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Mechanical significance of streptostyly in lizards

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The morphology of the lizard skull has been a subject of study for more than a century, particularly with respect to the morphology and function of the major jaw adducting muscles¹⁻⁵ and the mechanics of the moving parts⁶⁻⁹. It is possible that controversies surrounding the extent and timing of bone movement, muscle contraction and force generation will be resolved by techniques such as electromyography, cineradiography and measurement of bone strain^{3,10}. I present here data that facilitate a reconsideration of the function of the pterygoideus muscle, one of the two major jaw adducting muscles, and the mechanical significance of movements of the quadrate around the quadrate-squamosal joint. This movement, known as streptostyly⁶ occurs in all living lizards¹¹ and also characterises the earliest members of the order¹²⁻¹⁴. On the basis of my data I propose that streptostyly in lizards is a means by which the mechanical advantage of the pterygoideus muscle is increased, so that this muscle makes a major contribution to bite force.

The data were gathered from simultaneous electromyographic and cineradiographic experiments on *Varanus exanthematicus*, *Ctenosaura similis*, and *Tupinambis nigrapunctata*, cineradiographic experiments on *Uromastix aegyptius*, and strain gauge and force transducer recording of biting in *V. exanthematicus*. Materials and methods were essentially the

same as before for both cineradiography and electromyography¹⁰ and for strain gauge experiments¹¹. (Full details of the experiments will be reported elsewhere.)

Figure 1 shows the major morphological features to be discussed here. Of the three major jaw adducting muscles in lizards, the pterygoideus in particular has several interesting features. It is large; in *Varanus*, for example, it is 30-40% of the total adductor mass by weight; however, its mechanical advantage around the quadrate-mandibular joint is small. Generally it is not emphasised as an effective force producing muscle^{3,7-9} or it is assumed to be important only in initiating fast closing^{4,5}. Its position is such that in addition to any effect at the quadrate-mandibular joint, contraction will draw the lower end of the quadrate forward (protraction) relative to the quadrate-squamosal joint.

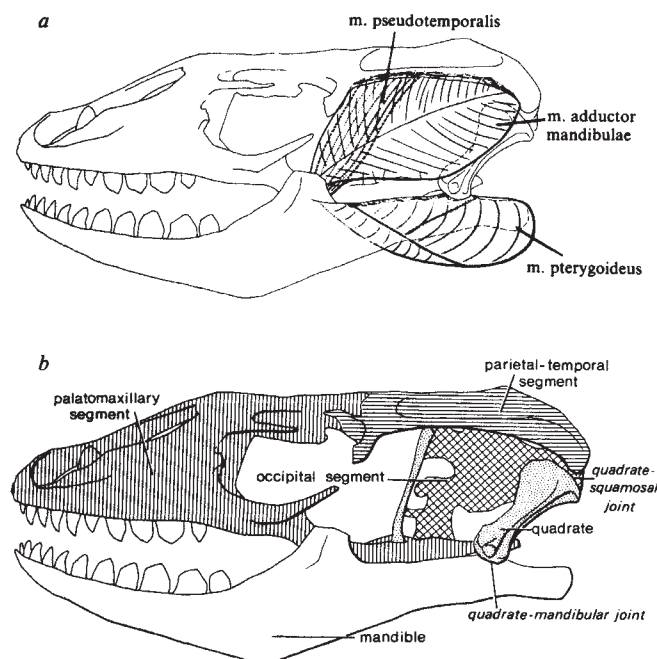


Fig. 1 *a*, Diagram of jaw adducting muscles in *Varanus exanthematicus*. The highly pinnate m. adductor mandibulae originates on the parietal-temporal and occipital units and inserts by means of a large central tendon on the coronoid region of the lower jaw. The m. pseudotemporalis originates from the anterior portions of the parietal-temporal and occipital units and inserts on the coronoid region medial to the m. adductor mandibulae. The m. pterygoideus originates on the pterygoid bone and passes ventrally and dorsally to insert on the ventral surface and retroarticular process of the mandible. This muscle, like the m. adductor mandibulae is highly pinnate, with many fibres running from the external aponeurosis of this muscle to the retroarticular process of the mandible. (For details see refs 1 and 2.) *b*, Bony structure of the lizard skull. The segments potentially important in lizard kinesis are indicated. Movement of the palato-maxillary unit relative to the parietal-temporal and occipital is termed mesokinesis, and that of the occipital unit relative to the parietal-temporal and palato-maxillary is termed metakinesis⁶. Movement of the quadrate at the quadrate-squamosal joint is termed streptostyly⁶. See text for further details.

Electromyographic recordings of the pterygoideus muscle show that it is active during all stages of feeding, including both ingestion cycles and 'power phases' when the animal is biting hard on food. Because it has been suggested that the pterygoideus is ineffective as a force-producing muscle, bite force was measured by stimulating this muscle electrically in an anaesthetised animal. The recordings indicated that the pterygoideus can produce a bite force at least equal to that of the adductor mandibulae externus.

Previous studies of streptostyly^{3,7-9,12,14} have, for the most part, assumed that it is most important during jaw opening and closing in cycles of ingestion and that its primary function is to produce anterior movement of the lower jaw. Further, movement of the quadrate is thought to be linked to movement of the maxillary segment.

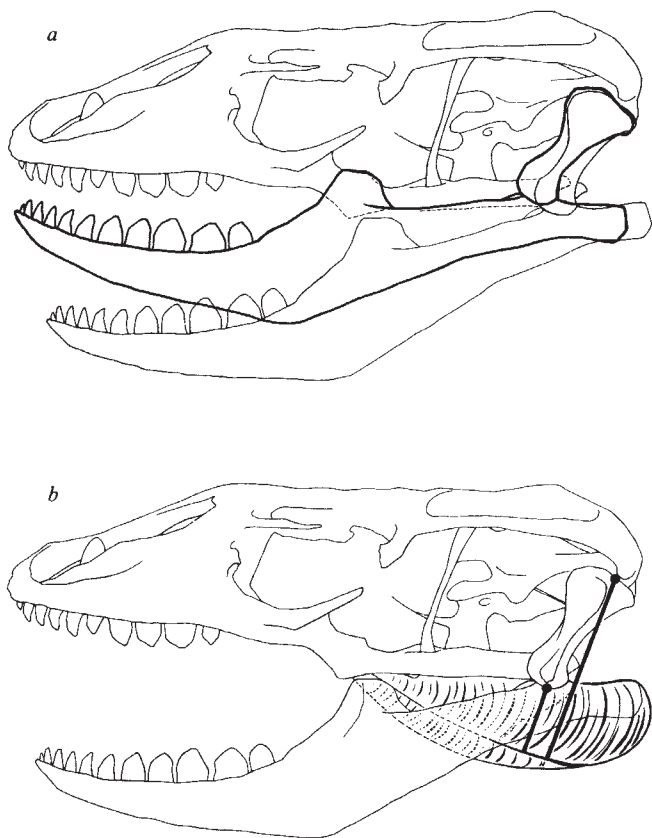


Fig. 2 *a*, Effect of quadrate protraction on gape. When the quadrate-mandibular joint is stabilised by contraction of any part of the adductor mass, movement at the quadrate-squamosal joint leads to jaw adduction. The dark line represents the result of 11° of quadrate protraction. *b*, Moment arms of the *m. pterygoideus* around the quadrate-squamosal and quadrate-mandibular joints. The mechanical advantage and thus potential contribution to bite force is almost four times as great when this muscle acts around the quadrate-squamosal joint.

Although the quadrate is potentially mobile in all the animals studied, streptostyly was observed only irregularly during cycles of jaw opening and closing in most animals. It was most regular in *Uromastix*. Streptostyly is independent of movement of the palato-maxillary segment (mesokinesis), as Throckmorton³ found. This would be predicted by careful morphological examination of fresh specimens. In lizards, a ligament binding the quadrate to the pterygoid bone restricts posterior movement of the quadrate, but no such ligament restricts forward movement. Streptostylic quadrates are found in fossil lizards before the appearance of mesokinesis¹²⁻¹⁴ and occur in animals with limited mesokinetic potential such as *Uromastix* and *Ctenosaura*. Streptostyly thus preceded and is independent of mesokinesis.

As an alternative to looking at the effect of streptostyly only when the jaw is opening, I note that protraction of the quadrate will close the jaws when the quadrate-mandibular joint is stabilised (Fig 2*a*). Contraction of any of the jaw adductors will lead to such stabilisation. In lizards, therefore, both the quadrate-mandibular and quadrate-squamosal joints are axes for jaw adduction. When an animal bites against resistance, little

movement occurs at either joint, but both joints act as fulcrum for the production of bite force. I suggest that streptostyly is not primarily important for the forward movement it imparts to the lower jaw, but for its contribution to jaw adduction during hard biting.

As pointed out above, contraction of the pterygoideus muscle leads to quadrate protraction. Figure 2*b* compares the mechanical advantage of the pterygoideus muscle around the quadrate-squamosal and quadrate-mandibular joints. Whereas the adductor mandibulae externus may produce power at the quadrate-mandibular joint, the pterygoideus muscle has primary mechanical advantage to act around the fulcrum at the quadrate-squamosal joint. Thus the significance of the quadrate-squamosal joint is that it allows a muscle with small mechanical advantage around the quadrate-mandibular joint to make a major contribution to bite force.

There are several functional implications of this two-joint jaw suspension system. During ingestion of food, when wide gape and fast movements are primarily important, movement around the quadrate-mandibular joint allows wide excursion of the mandible. However, when the animal bites against resistance, muscles act around both the quadrate-mandibular and quadrate-squamosal joints. The position of the pterygoideus muscle is such that it may produce fast closing around the quadrate-mandibular joint during ingestion and powerful adduction around the quadrate-squamosal joint during hard biting. With this two-joint system, the pterygoideus muscle avoids potential mechanical compromises between power and speed. Further, lizards with large pterygoideus muscles develop an effective force-producing muscle without interfering with gape. Increasing the size of the pseudotemporalis muscle would, for example, increase potential bite force, but the position of this muscle is such that gape would be severely restricted.

This scheme applies generally to all living lizards because they all have streptostylic quadrates, but it must be carefully applied to fossil reptiles. The rhynchocephalian, *Glevosaurus*, and the prolacertilians¹² possess an interrupted lower temporal arcade, but fused quadrate-ptyergoid and quadrate-squamosal junctions. In neither of these forms is the quadrate free swinging or streptostylic. Only in the true Squamata, including early forms such as the Paliguanidae¹⁴, *Kuehneosaurus*¹² and *Icarosaurus*¹³ was the streptostylic condition achieved. In the lepidosaurian reptiles the lower temporal arcade has been lost at least three times independently; however, in many forms this loss is not related to quadrate mobility. As well as lizards, snakes and birds also have streptostylic quadrates, but their skull morphology is such that in morphological and functional detail there is little real equivalence in the three systems, and the scheme applied here does not apply.

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Selective reconstitution of nude mice with long-term cultured and cloned specific helper T cells

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An antibody response is the end result of complex interactions among T cells, adherent cells and B cells¹⁻³. An understanding of the interactions involved has proved difficult as pure populations of these cells have not been available. By making use of T-cell growth factor^{4,5}, we were able to grow normal helper T cells specific for heterologous erythrocytes⁶. Because specificity and mechanism of action of these cells had been demonstrated solely in culture, we sought to establish their competence in the whole animal. We have therefore examined here whether antigen-specific helper T cells, maintained in culture over long periods, would enable syngeneic nude mice to respond to T-cell-dependent antigens. The results show that specific helper T cells, propagated in serum-free medium *in vitro* for up to 15 months, can selectively and specifically reconstitute syngeneic C57BL/6J *nu/nu* mice. Depending on the specificity of the injected helper T cells, such nude mice could respond to sheep red blood cells (SRC) but not to horse red blood cells (HRC) and vice versa. The magnitude of the response was comparable to that of normal mice and could exceed it by almost 10-fold, depending on the source and number of injected helper T cells.

Table 1 documents the *in vitro* helper activity of the long-term cultured, cloned and subcloned helper T cells at the time of the present report. 10^3 to $10^{3.5}$ of these cells provided maximal T-cell help if added to 2×10^5 nude spleen cells in the presence of homologous antigen. A higher input of helper T cells rendered the culture medium acid and lowered the numbers of IgM plaque-forming cells (PFC). No PFC were induced in response to the unrelated antigen, indicating that these T cells were specific and that no polyclonal activation had occurred by day 5 of culture.

Injection of uncloned helper T cells (cultured over 3–4 months) with homologous antigen into syngeneic nude mice, induced a specific antibody response in these animals (Table 2). 5×10^6 HRC-specific uncloned helper T cells (LTC 15) gave rise to about five times as many direct PFC as could be expected in a

primary response of normal mice 5 days after immunisation⁷. The simultaneous injection of the uncloned SRC-specific helper T-cell line LTC 25 and antigen (Table 2) gave rise to almost 10^6 direct PFC on day 5. The response to the unrelated antigen hardly exceeded background even when both antigens were injected at the same time (LTC 3, Table 2).

The unusually high response may reflect the lack of regulatory mechanisms in the nude mouse, such as 7S feedback inhibition⁸ or the absence of suppressor T cells⁹. This is also indicated by the kinetics of the response. In contrast to normal mice, there was no sharp decrease in the number of PFC beyond day 5 after immunisation. Most reconstituted mice showed high or even increasing numbers of PFC up to day 7 after immunisation. Beyond that, the number of PFC varied considerably in individual mice before returning to background around 10 days after inoculation.

We have not determined what fraction of the injected helper T cells homes to the sites of antibody formation in the spleen. This aspect has an even stronger bearing on our reconstitution experiments with clones of helper T cells kept in continuous culture for up to 15 months. Table 2 shows results obtained with two of our clones and with one subclone (18/33/3). Although the number of PFC is somewhat lower than that obtained with uncloned helper T cells, it is still entirely comparable with the response of a normal mouse. The high degree of specificity remains unchanged. The antigen-independent anti-HRC response of subclone 18/33/3 (Table 2, last line) is under investigation.

The fact that a single clone of helper T cells can reconstitute a primary antibody response of normal magnitude raises the question of whether assumed restrictions in T-cell–B-cell interaction, operating at the level of idiotypes¹⁰ or on a subset of cells¹¹, are compatible with our findings. It should be remembered that a nude spleen (on average 8×10^7 cells) contains at least 20,000 B-cell precursors, which can recognise the antigen SRC and are susceptible to T-cell help¹². This minimal estimate does not account for antigen-specific recruitment of lymphocytes¹³. As 10^3 PFC can arise *in vitro* from one precursor within 5 days¹², it is obvious that the induction of less than 1% of B-cell precursors would be sufficient to give rise to about 10^5 PFC by day 5 after immunisation. Idiotypic analysis of the antibodies in normal and clonally reconstituted mice may provide critical information. Our preliminary results suggest that significant differences at the antibody level may exist between such mice. Thus, although the serum of the clonally reconstituted mice (18/33/3, Table 2) gave a haemagglutination end point when diluted 1 in 28,000, the average titres were significantly lower in mice reconstituted with uncloned specific helper T cells, even

Table 1 *In vitro* helper activity of long-term cultured, cloned or subcloned specific helper T cells at the time of *in vivo* reconstitution experiments

No. of T cells added per culture	Designation of T-cell line (LTC), clone or subclone (specificity given in parentheses)									
	LTC 15 (T _{HRC})		LTC 3 (T _{SRC})		LTC 25 (T _{SRC})		Clone 18/19 (T _{HRC})		Clone 1/25 (T _{SRC})	
	Antigen present in the culture (no. of PFC per culture directed against this antigen)									
	HRC	SRC	HRC	SRC	HRC	SRC	HRC	SRC	HRC	SRC
10^4	ND		ND		0	10,973 ± 11,422	ND		ND	2,470 ± 1,543
$10^{3.5}$	3,900 ± 2,561	0	0	6,540 ± 2,907	0	11,440 ± 5,312	7,260 ± 3,710	0	0	2,520 ± 3,710
10^3	6,440 ± 2,850	0	0	9,410 ± 5,849	0	3,620 ± 825	8,740 ± 3,355	0	0	3,910 ± 1,748
$10^{2.5}$	2,195 ± 1,440	0	0	1,455 ± 681	ND	ND	890 ± 382	0	0	1,110 ± 167
10^2	40 ± 14	0	0	25 ± 21	ND	ND	0	0	137 ± 145	ND

Helper T cells were tested for helper activity and specificity in a specific *in vitro* helper assay¹². 2×10^5 spleen cells from C57BL/6J *nu/nu* mice were cultured in a volume of 0.2 ml in flat bottom Falcon II tissue culture plates in the presence of 5×10^5 SRC or HRC and the indicated number of helper T cells. The culture medium was IMDM¹⁵, supplemented with bovine serum albumin (1 mg ml^{-1}), human transferrin ($30 \text{ } \mu\text{g ml}^{-1}$), soybean lipids ($100 \text{ } \mu\text{g ml}^{-1}$) and cholesterol ($50 \text{ } \mu\text{g ml}^{-1}$)^{12,15,16}. PFC were enumerated after 5 days in a modified Jerne plaque assay¹⁷. They are expressed as direct PFC per culture $\pm$ standard deviation of the mean. The helper T cells, specific for SRC or HRC (T_{SRC}, T_{HRC}) were obtained from spleens of lethally irradiated mice which had been reconstituted 7 days previously with thymocytes and injected with SRC or HRC (*in vivo* activated helper T cells (refs 12, 18)). They were cultured in the presence of antigen in Falcon 3013 tissue culture flasks in the same medium as described for the *in vitro* helper assay at a concentration of 10^6 activated T cells per ml. Antigen and irradiated syngeneic peritoneal cells (3,300R) were replenished at 1–2-week intervals. After 2–3 months of culture vigorous growth of cells was induced by supplementing the medium with partially purified T-cell growth factor⁵. The T-cell lines LTC15, 3 and 25 were subcultured and grown for another 3–4 months in TCGF-supplemented medium in the absence of antigen and peritoneal cells. Such T-cell lines were cloned (clone 18/19 and 1/25) and subcloned (18/33/3) in conditions of limiting dilution with a cloning efficiency of 5–20% (ref. 6). None of the T cells was exposed to antigen 3–4 months before the experiment.

Table 2 Specific reconstitution of C57BL/6J *nu/nu* mice with long-term cultured or cloned helper T cells specific for HRC (T_{HRC}) or SRC (T_{SRC})

T cells (line, clone or subclone)	No. of T cells injected per mouse	No. of mice per group	Antigen injected	PFC per spleen directed against HRC	PFC per spleen directed against SRC
LTC 15 (T _{HRC})	Nil	5	HRC	1,130 ± 277	304 ± 210
	10 ⁵	4	HRC	2,350 ± 290	ND
	10 ⁶	5	HRC	53,680 ± 11,356	ND
	5 × 10 ⁶	4	HRC	580,000 ± 76,302	272 ± 166
LTC 3 (T _{SRC})	4 × 10 ⁶	2	SRC + HRC	575 ± 412	268,450 ± 26,474
LTC 25 (T _{SRC})	5 × 10 ⁶	4	SRC	622 ± 398	940,250 ± 52,143
	5 × 10 ⁶	4	HRC	1,746 ± 1,308	1,345 ± 1,163
18/19 Clone (T _{HRC})	2 × 10 ⁶	2	HRC	52,727 ± 14,978	274 ± 134
1/25 Clone (T _{SRC})	5 × 10 ⁶	4	SRC	485 ± 95	64,377 ± 4,919
18/33/3	5 × 10 ⁶	4	HRC	159,100 ± 11,172	460 ± 254
Subclone (T _{HRC})	5 × 10 ⁶	4	SRC	14,380 ± 1,951	965 ± 306

C57BL/6J *nu/nu* mice, 9th or 10th backcross (Institute for Biologisch-Medizinische Forschung, Füllinsdorf, Switzerland) were injected intravenously with long-term cultured or cloned helper T cells, together with 5 × 10⁶ SRC and/or HRC. Five days later the number of direct PFC in the spleen, directed against either antigen, was determined in a modified Jerne plaque assay¹⁷.

though the number of PFC per spleen was considerably higher (for example, LTC 25, Table 2).

At present, we know little about the long-term fate of these injected helper T cells *in vivo*. Boosting of mice which had been reconstituted with the SRC-specific clone 1/25 and primed 21 days earlier, gave rise to even higher numbers of PFC against the homologous antigen. This suggests that these helper T cells survive in the recipient animal. As nude mice cannot generate T-cell growth factor¹⁹, an absolute requirement for T-cell proliferation^{4,5}, the reconstituting cells may revert there to a resting state. In the experiments presented here, we studied a few clones only, and have not observed a significant switch to IgG production. By using various clones and immunisation schedules, we should be able to decide whether the same helper T cell can provide help for various antibody classes.

Our results show that helper T cells reveal the same degree of specificity *in vitro* and *in vivo*. *In vitro*, these helper T cells are H-2 restricted, the restriction mapping to the left of the I-B region¹⁴. Reconstitution of T-cell-deficient mice with T cells selected and passaged in culture provides a new tool for studying

the function and regulation of specific helper T cells, and should prove especially useful in discriminating between useful and misleading *in vitro* phenomena.

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Vitamin B₁₂ synthesis by human small intestinal bacteria

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In man, physiological amounts of vitamin B₁₂ (cyanocobalamin) are absorbed by the intrinsic factor mediated mechanism exclusively in the ileum¹. Human faeces contain appreciable quantities of vitamin B₁₂ or vitamin B₁₂-like material presumably produced by bacteria in the colon², but this is unavailable to the non-coproagic individual. However, the human small intestine also often harbours a considerable microflora^{3–6} and this is even more extensive in apparently healthy southern Indian subjects⁶. We now show that at least two groups of organisms in the small bowel, *Pseudomonas* and *Klebsiella* sp., may synthesise significant amounts of the vitamin.

Bacteria were obtained by aspiration from the jejunum and ileum of healthy southern Indian control subjects, and patients with tropical sprue. All subjects gave their informed consent to the study and all had normal vitamin B₁₂ absorption. Full details of the intubation, bacteriological techniques and vitamin B₁₂ absorption tests have been described elsewhere⁶. Representative isolates of different groups of aerobic and anaerobic organisms were grown in the medium described by Nishio *et al.*⁷ modified to support the growth of fastidious organisms⁸.

Vitamin B₁₂ activity was measured in the presence of potassium cyanide by microbiological assay using *Euglena gracilis* Z strain⁹ and *Ochromonas malhamensis*¹⁰ as the test organisms. Descending paper chromatography was carried out in sec-butanol/acetic acid/water¹¹ and bioautography by the method of Linnel *et al.*¹².

Using the *Euglena gracilis* assay, the following groups of organisms were found to produce no detectable vitamin B₁₂-like material (the numbers in parentheses indicate the number of strains tested): enterobacteria (10), *Escherichia coli* (17), streptococci (18), *Neisseria* (5), staphylococci (4), aerobic lactobacilli (12), bifidobacteria (11), and *Bacteroides* (8). With the same assay, five groups of organisms produced considerable amounts of vitamin B₁₂-like material (Table 1). A subsample of these five groups of organisms were also tested using the *Ochromonas* assay (Table 1). Since the growth response of *Ochromonas* is more specific for vitamin B₁₂ than that of *Euglena*¹⁰, the material produced by the clostridia, *Veillonella* and *Fusobacterium* presumably consists of vitamin B₁₂ analogues, whereas that produced by *Pseudomonas* and *Klebsiella* probably is biologically active vitamin B₁₂. This was further supported by chromatography and bioautography of the material produced by *Pseudomonas* which gave an *R_f* value identical to that of cyanocobalamin. A comparison of the whole broth and bacteria-free filtrates showed that in the case of *Klebsiella* the vitamin B₁₂ activity was associated with the bacterial bodies but that with *Pseudomonas* the activity was principally free in the supernatant.

It has been shown that free intrinsic factor is often present in the lumen of the small intestine¹³. If these organisms also produce vitamin B₁₂ *in vivo*, the free vitamin B₁₂, or that bound to bacteria, after undergoing liberation by digestive enzymes, could bind with the free intrinsic factor and, if absorbed, contri-

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Table 1 Vitamin B₁₂ activity of bacteria isolated from jejunal aspirates

	<i>Euglena gracilis</i> assay		<i>Ochromonas</i> <i>malhamensis</i> assay	
	No. of strains tested	Vitamin B ₁₂ - like activity ($\mu\text{g l}^{-1}$ culture)	No. of strains tested	Vitamin B ₁₂ - like activity ($\mu\text{g l}^{-1}$ in culture)
		Mean Range		Mean Range
<i>Pseudomonas</i>	7	15.0 13.0–17.3	3	14.0 10.8–18.8
<i>Klebsiella</i>	10	2.9 1.5–7.2	2	3.2 1.4–5.0
Lecithinase- positive clostridia	7	14.0 5.2–37.9	2	0.1 0–0.2
<i>Veillonella</i>	13	14.3 7.2–40.0	3	0.6 0–0.9
<i>Fusobacteria</i>	6	26.6 9.2–36.0	3	0 0–0

bute significantly to the vitamin B₁₂ intake of the individual. For example, 10 ml of a culture of *Pseudomonas* would provide 0.14 μg of the vitamin which is more than the minimum daily dose of 0.1 μg which will produce a haematological response in subjects with vitamin B₁₂ deficiency megaloblastic anaemia¹⁴.

The vitamin B₁₂ analogues produced by the bacterial isolates were not identified further. Those with no alteration in the corrin ring or benzimidazole moiety can also combine with free intrinsic factor^{15,16}. However, since the amount of intrinsic factor passing through the intestine is in considerable excess of that needed to absorb the minimum daily vitamin B₁₂ requirements¹³, the appropriate analogues would have to be present in considerable amounts significantly to reduce the amount of intestinally produced vitamin B₁₂ absorbed by normal subjects.

The role of intraluminal small intestinal bacteria in human vitamin B₁₂ metabolism appears complex. Subjects with small intestinal bacterial overgrowth associated with a stagnant bowel syndrome, frequently have vitamin B₁₂ malabsorption^{17,18}. This malabsorption may often be reversed by the administration of broad spectrum antibiotics¹⁸, suggesting that the defect is closely related to the bacterial overgrowth. However, the precise mechanism for the vitamin B₁₂ malabsorption in these subjects is not clear. Various mechanisms have been suggested, including the bacterial uptake of vitamin B₁₂ (ref. 19) or the intrinsic factor B₁₂ complex^{20,21}; alteration of the IF-B₁₂ complex²²; the production of toxins²³ which may affect the binding of the IF-B₁₂ complex to the receptor²⁴; and the production of large amounts of vitamin B₁₂ analogues²⁵ blocking vitamin B₁₂ absorption. In apparently healthy southern Indian subjects with normal vitamin B₁₂ absorption there is frequently considerable bacterial colonisation of the upper small intestine⁶, but the degree of bacterial overgrowth is not as great as in patients with a stagnant bowel syndrome with vitamin B₁₂ malabsorption²⁶. This fact, and the finding in the present study that some organisms produce predominantly vitamin B₁₂-like material (*Ochromonas malhamensis* growth factor) while others produce only vitamin B₁₂ analogues (*Euglena gracilis* but not *Ochromonas malhamensis* growth factor), suggests that the quantity and type of the luminal flora may be important in determining the role of the intestinal bacteria in the vitamin B₁₂ ecology of the individual.

In the light of the findings reported here, it is of interest that vitamin B₁₂ deficiency among vegetarian Indians has been found with relatively high frequency in Indian immigrants in England^{27–30}, whereas it is uncommon among the same people in India with identical dietary patterns³¹. It is possible that in India these individuals have a bacterial flora in the small intestine which provides a significant proportion of their daily requirements of vitamin B₁₂, but when they move to a more protected environment the bacterial flora reverts to that characteristic of subjects living in western countries with little or no resident flora in the upper small bowel³, and individuals lose their endogenous source of vitamin B₁₂ and therefore become more prone to develop overt vitamin B₁₂ deficiency.

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A factor(s) in *Klebsiella* culture filtrates specifically modifies an HLA-B27-associated cell-surface component

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We have previously shown^{1,2} that a serum raised against certain isolates of *Klebsiella pneumoniae* lyses the lymphocytes of HLA-B27-positive patients with ankylosing spondylitis (AS) but not of B27-positive or B27-negative healthy controls. These observations suggested that some *Klebsiella* antigens cross-react with a gene product intimately associated with B27 or possibly with 'modified' B27 (refs 1, 3) in patients with AS. It seems likely therefore, that some *Klebsiella* antigens play a role in the pathogenesis of AS, perhaps by specifically modifying a B27-associated cell-surface marker. We have now examined the influence of culture filtrates from three *Klebsiella* isolates on lymphocytes from B27-positive and B27-negative healthy individuals. We report that 24-h culture filtrates of *Klebsiella* K43 (previously *Klebsiella* F19) contain a factor(s) capable of specifically modifying the sensitivity to anti-*Klebsiella* K43 serum of B27⁺AS⁺ lymphocytes (that is, cells not lysed by anti-*Klebsiella* K43 serum): 'modified' B27⁺AS⁺ lymphocytes are now serologically similar to the cells of B27-positive patients with AS (B27⁺AS⁺). The failure of anti-*Klebsiella* F10 and of anti-*Escherichia coli* sera to lyse lymphocytes, which have been incubated with the corresponding culture filtrates, excludes a nonspecific lysis by the anti-bacterial sera of target lymphocytes bearing bacterial antigens randomly distributed on their surface. Therefore, the data are compatible with a specific transformation by a *Klebsiella* K43-derived soluble factor of a B27-associated lymphoid cell component.

Bacterial cultures were isolated from faecal and urine samples (*Klebsiella pneumoniae* K43, *Klebsiella* isolates F10, F12 and 462; *E. coli*) and from a wound swab (*Staphylococcus* sp.). Bacteria were grown in Tryptone soya broth (TSB, Oxoid) and killed by adding 0.25 ml of 40% formaldehyde to a 20 ml suspension of the organisms ($\sim 10^9$ per ml) in nutrient broth. The formalin-killed bacteria were washed twice with sterile 0.9% NaCl and stored at -20°C . Antisera were raised in rabbits (3–5 kg) against formalin-killed suspensions of the bacteria as previously described¹. Briefly, the animals were injected intravenously (i.v.) with about 10^9 bacteria in normal saline (2 ml) and 2 weeks later, intramuscularly with 10^{10} organisms in 2 ml complete Freund's adjuvant (Difco). Ten days later, the rabbits were boosted i.v. with 1 ml of bacterial suspension (10^8 – 10^9 organisms) in normal saline. Two weeks after the last injection, the animals were bled and the serum stored at -20°C .

The results in Table 1 show that the lymphocytes of B27⁺AS[−] individuals, incubated for 24 h in TSB or with culture filtrates of *Klebsiella* F10, F12 or with *E. coli*, are not lysed by anti-*Klebsiella* K43 serum. Similarly, anti-*Klebsiella* K43 serum is

not cytotoxic for the lymphocytes of B27[−]AS[−] or of B27[−]AS⁺ individuals following a 24-h incubation with any of the bacterial culture filtrates. On the other hand, the strong reactivity to anti-*Klebsiella* K43 serum of B27⁺ cells from patients with ankylosing spondylitis (by Rome and New York criteria)⁴ is preserved even when these cells are cultured for 24 h in the four different culture filtrates. By contrast, B27⁺AS[−] lymphocytes, incubated with culture supernatants of *Klebsiella* K43, become susceptible to lysis by anti-*Klebsiella* K43 serum but not by antisera to *Klebsiella* isolates F10, 462 or to *E. coli* and *Staphylococcus* sp. Furthermore, no 'extra' serologically-defined specificities are detected when *Klebsiella* K43-incubated B27⁺AS[−] cells were tested against a panel of antisera defining 13 HLA-A and 20 HLA-B specificities (results not shown). The transformation of B27⁺AS[−] lymphocytes appears to be a specific event and not a consequence of randomly-attached *Klebsiella* K43 determinants on the surface of B27⁺AS[−] cells. This conclusion is supported by the failure of anti-*Klebsiella* F10 serum to lyse lymphocytes incubated with culture filtrates of *Klebsiella* F10. As B27[−]AS[−] and B27[−]AS⁺ lymphocytes are not

Table 1 Culture filtrates of *Klebsiella* K43* specifically modify a B27-associated cell surface component on the lymphocytes of B27-positive healthy individuals but not on the cells of B27-negative healthy controls; the lymphocytes of B27-negative patients with AS are also not modified

Incubation	Serum	Target lymphocytes			
		B27 ⁺ AS [−]	B27 [−] AS [−]	B27 [−] AS ⁺	B27 ⁺ AS ⁺
Nutrient broth	Anti-B27	51	0	4	60
	Anti- <i>Klebsiella</i> K43	2	1	2	81
	Anti- <i>Klebsiella</i> F10	10	0	0	1
	Anti- <i>Klebsiella</i> 462	5	0	NT	6
	Anti- <i>E. coli</i>	4	0	18	5
	Anti- <i>Staphylococcus</i>	3	8	NT	NT
Culture filtrate of <i>Klebsiella</i> K43	Anti-B27	49	5	0	69
	Anti- <i>Klebsiella</i> K43	71	9	7	85
	Anti- <i>Klebsiella</i> K10	7	12	0	1
	Anti- <i>Klebsiella</i> 462	7	9	1	1
	Anti- <i>E. coli</i>	9	4	0	5
	Anti- <i>Staphylococcus</i>	4	6	NT	NT
Culture filtrate of <i>Klebsiella</i> F10	Anti-B27	58	4	7	71
	Anti- <i>Klebsiella</i> K43	11	4	7	71
	Anti- <i>Klebsiella</i> F10	3	4	7	0
	Anti- <i>Klebsiella</i> 462	10	4	NT	NT
	Anti- <i>E. coli</i>	9	5	4	0
	Anti- <i>Staphylococcus</i>	NT	0	NT	NT
Culture filtrate of <i>Klebsiella</i> F12	Anti-B27	65	2	1	52
	Anti- <i>Klebsiella</i> K43	9	7	7	63
	Anti- <i>Klebsiella</i> F10	0	3	2	0
	Anti- <i>Klebsiella</i> 462	3	2	1	1
	Anti- <i>E. coli</i>	7	3	0	7
	Anti- <i>Staphylococcus</i>	0	NT	NT	NT
Culture filtrate of <i>E. coli</i>	Anti-B27	60	2	16	91
	Anti- <i>Klebsiella</i> K43	10	5	14	85
	Anti- <i>Klebsiella</i> F10	12	4	7	6
	Anti- <i>Klebsiella</i> 462	6	7	16	16
	Anti- <i>E. coli</i>	12	9	11	13
	Anti- <i>Staphylococcus</i>	1	0	NT	NT

Peripheral blood lymphocytes were obtained from groups of B27-positive normal controls (B27⁺AS[−], $n = 19$), B27-negative normal controls (B27[−]AS[−], $n = 17$), B27-positive patients with ankylosing spondylitis (B27⁺AS⁺, $n = 6$) and from B27-negative patients with ankylosing spondylitis (B27[−]AS⁺, $n = 3$). The cells were separated from heparinised blood by centrifugation in Ficoll-Paque (Pharmacia) for 25 min at 580g. The leukocyte band was removed and washed with RPMI 1640 medium (Flow) containing 2 ml heparin ($1,000 \text{ units ml}^{-1}$), 10 ml IM HEPPES (Sigma) buffer, 5 ml L-glutamine (200 mM) and 2 ml penicillin-streptomycin ($5,000 \text{ IU}$, $5,000 \text{ } \mu\text{g ml}^{-1}$; Flow) per 500 ml of RPMI medium. The leukocytes were suspended in RPMI medium containing 10% human AB serum and centrifuged for a further 10 min at 180g. The cells were then resuspended in RPMI medium containing 20% human AB serum and adjusted to a cell concentration of 2.5×10^6 mononuclear cells per ml of medium. The incubation mixture consisted of 3.8 ml of cell suspension in RPMI containing 20% human AB serum and either 0.2 ml of TSB (Oxoid; diluted 1:2 with Dulbecco phosphate buffer (DPB) just before use) or 0.2 ml of culture filtrate (diluted 1:2 with DPB). Higher concentrations of culture filtrate were non-specifically cytotoxic to human leukocytes. Both the TSB and the culture filtrates were Millipore-filtered before dilution with DPB. The lymphocytes were incubated with TSB or with the various culture filtrates at 37°C for 24 h. They were then washed twice with RPMI containing 10% human AB serum and resuspended at a concentration of 3×10^6 cells per ml. About 6×10^6 lymphocytes, suspended in 2 ml of RPMI containing 10% human AB serum, were labelled with $100 \text{ } \mu\text{Ci}$ of ^{51}Cr (0.1 ml ; 200 – $500 \text{ } \mu\text{Ci } \mu\text{g}^{-1}$, Amersham) for 60 min at 37°C . The cells were then washed twice with RPMI + 10% AB serum and resuspended at a concentration of 3×10^6 cells per ml. One hundred μl of the ^{51}Cr -labelled cell suspension were added to 10×75 -mm plastic tubes and incubated with $100 \text{ } \mu\text{l}$ of antiserum at 24°C for 30 min. Rabbit complement ($100 \text{ } \mu\text{l}$) was then added to each tube and the incubation continued for a further 60 min. The cells were centrifuged at $2,000 \text{ r.p.m.}$ for 15 min and $100 \text{ } \mu\text{l}$ of the supernatant was counted in a Packard autogamma scintillation spectrometer. The amount of radioactivity in the samples was compared with radioactivity present in an equal volume of the supernatant from tubes containing cells in medium plus complement only, and with that present in tubes containing cells which had been lysed by the addition of $200 \text{ } \mu\text{l}$ of Nonidet-P40. The results are expressed as the percentage of maximum ^{51}Cr released, which was calculated as follows:—

$$^{51}\text{Cr release \%} = 100 \times \frac{(\text{Radioactivity released by antiserum}) - (\text{Radioactivity released in absence of antiserum})}{(\text{Radioactivity released by NP-40-solubilised cells}) - (\text{Radioactivity released in absence of antiserum})}$$

The results shown are of a representative experiment on the cells of single individuals for each category. All sera were tested undiluted. NT, not tested.

* *Klebsiella* K43 is a standard capsular designation; *Klebsiella* F10 and 462, antisera to which do not lyse B27⁺AS⁺ lymphocytes, are two different isolates which have not yet been serotyped.

Table 2 Reactivity to anti-*Klebsiella* K43 serum of B27⁺AS⁻, B27⁻AS⁻ and B27⁻AS⁺ lymphocytes incubated with culture filtrates of *Klebsiella* K43

Diagnosis	B27-positive	No. transformed no. tested
Normal	Yes	17/19
Normal	No	0/17
Definite AS	No	0/3

Diagnosis was based on Rome and New York criteria. Cells are regarded as transformed if at least 50% are lysed by anti-*Klebsiella* K43 serum after incubation with K43 filtrate.

transformable by any of the *Klebsiella* culture filtrates tested, it appears that B27 or the product of a closely-linked gene is a specific target for a *Klebsiella* K43-derived factor(s).

Table 2 summarises the data on the reactivity to anti-*Klebsiella* K43 serum of B27⁺AS⁻, B27⁻AS⁻ and B27⁻AS⁺ lymphocytes incubated with culture filtrates of *Klebsiella* K43. The results indicate that none of the cells of B27-negative patients with AS nor of B27-negative normal controls is transformable by the *Klebsiella* K43-derived culture supernatant. However, two of the nineteen B27⁺AS⁻ cells have failed to be 'converted' by the *Klebsiella* K43 culture filtrate. It is apparent (Table 1 and Table 2) that only some strains of *Klebsiella* can trigger the specific transformation of B27⁺AS⁻ cells and it is possible that some individuals carry a 'subtype' of B27 which lacks a hypothetical receptor required to specifically bind a *Klebsiella* K43 antigenic determinant. This effect has also been demonstrated with another serotype of *Klebsiella* (K21; serotyped by Dr. I. Ørskov), indicating that the cross-reactivity of B27⁺AS⁺ cells with some strains of *Klebsiella* is not related to the capsular serotype of the organism.

Although the chemical nature and mechanism of action of *Klebsiella*-derived factors are not yet understood, preliminary results indicate that they are non-dialysable but heat labile at 56 °C for 30 min. (results not shown).

Bacterial products have generally been shown to have a nonspecific influence on the immune response. Thus, culture filtrates and cell wall-rich fractions of *Listeria monocytogenes* contain substances that are mitogenic for B lymphocytes *in vitro*^{5,6} while *in vivo* they, (1) function as immunologic adjuvants^{7,8}, (2) activate macrophages⁵ and (3) induce a transitory state of nonspecific resistance to infection by live *Listeria*^{5,9}.

Furthermore, lipopolysaccharide, dextran sulphate, mycobacteria¹⁰ and peptidoglycan¹¹ have been shown to be B cell-specific polyclonal activators of mouse lymphocytes. To our knowledge, the studies reported here represent the first demonstration that bacterial antigens may have a specific effect on HLA-associated gene products. It is tempting to suggest that the modification by environmental agents of specific cell surface components is an important step in the pathogenesis of the HLA-linked seronegative arthropathies.

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Covalent attachment of a progestational steroid to chick oviduct progesterone receptor by photoaffinity labelling

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The chick oviduct progesterone receptor has been characterised and purified by us^{1–3} and shown to consist of a mixture of two hormone-binding proteins^{4,5}. These two proteins have equivalent ligand-binding sites, to which progesterone and other progestational steroids bind with high affinity ($k_{\text{dis}} = 10^{-9}$ M)^{6,7}. Due to the noncovalent nature of this interaction, analysis of the proteins in denaturing or nonequilibrium conditions has always been hampered by ligand-protein dissociation during the experiments ($t_{1/2} = 12$ h at 0 °C, 25 min at 37 °C)⁶. Efforts to use covalent attachment of steroids by alkylation or photoactivation have been largely unsuccessful, both for this protein and for other receptors⁸. However, covalent labelling of steroid isomerases⁹ and an androgen-binding protein¹⁰ has been obtained in reasonable yields using photoactivation of keto steroids containing multiple double bonds in conjugation with the ring-A ketone. We now report the first successful covalent attachment of a radiolabelled steroid to its receptor protein. The ligand chosen was a synthetic progestin, 17 α ,21-dimethyl-19-nor-pregn-4,9-diene-3,20-dione (R5020; Roussel-Uclaf)¹¹. R5020 is available as the tritiated 17 α -methyl derivative (≥ 50 Ci mmol⁻¹) and has been used extensively for measuring mammalian progesterone receptors in normal^{12,13} and neoplastic tissues^{14,15}. This steroid was chosen for the photoaffinity study because its absorption maximum is at 320 nm, thereby allowing its activation by UV light at wavelengths above 300 nm, where protein damage would be minimised. The method should be generally applicable to other progestin receptors.

Oviduct cytosol from immature, oestrogenised chicks was prepared in buffer A (10 mM Tris-HCl, pH 7.4, 1 mM Na₂EDTA, 12 mM (1-thioglycerol) at 4 ml buffer per g tissue, as described previously¹⁶. The progesterone receptors were labelled with ³H-R5020 at 2×10^{-8} M for 2 h at 0 °C. Then the hormone-receptor complexes were precipitated at 30% saturation of ammonium sulphate in buffer A and redissolved in a volume of buffer A equal to the original cytosol volume. Equilibrium binding studies showed R5020 binding to the progesterone receptor, as determined by (1) competition and displacement by authentic progesterone, (2) labelling of the same number of binding sites in the preparation by either labelled R5020 or progesterone and (3) chromatographic analysis of R5020-receptor complexes by DEAE-cellulose, gel filtration and sucrose-gradient ultracentrifugation. In all these tests, R5020-receptor complexes were indistinguishable from the complexes formed by authentic progesterone. Finally, binding of ³H-R5020 to nonreceptor proteins (nonspecific weak absorption) was less than 5%.

Redissolved complexes were then photolysed at 0 °C in an ice bath using an 85-W low-pressure mercury vapour lamp (General Electric H85A3). Samples of receptor were placed in a beaker covered by a borosilicate glass plate to absorb UV below 300 nm. Aliquots (1.0 ml) were removed at intervals for up to 2 h.

Samples were tested initially for covalent steroid coupling by (1) addition of an equal volume of ice-cold 10% (w/v) of trichloroacetic acid (TCA). Precipitated protein was redissolved and counted for ³H. There was a time-dependent increase in precipitable ³H during UV illumination. Covalency was further

assessed by (2) vigorous, repeated (five times) extraction with a 20-fold excess of diethyl ether, (3) gel filtration of irradiated samples in 5.0 M urea and (4) dialysis of the complexes against 1% (w/v) SDS. In all four types of test, UV-ray irradiation for 60 min resulted in optimal coupling of ^3H -R5020 to acid-insoluble material with a nominal coupling efficiency of 10–20% of the input ligand. Thus, the photoactivation reaction was indeed resulting in covalent attachment of the steroid to protein. We used electrophoresis in denaturing conditions by the method of Laemmli^{2,3,17} to determine whether ^3H -R5020 was, in fact, attached to the desired receptors.

The results of these experiments are shown in Fig. 1. In *a*, three equivalent cytosol preparations (1.0 ml each) were

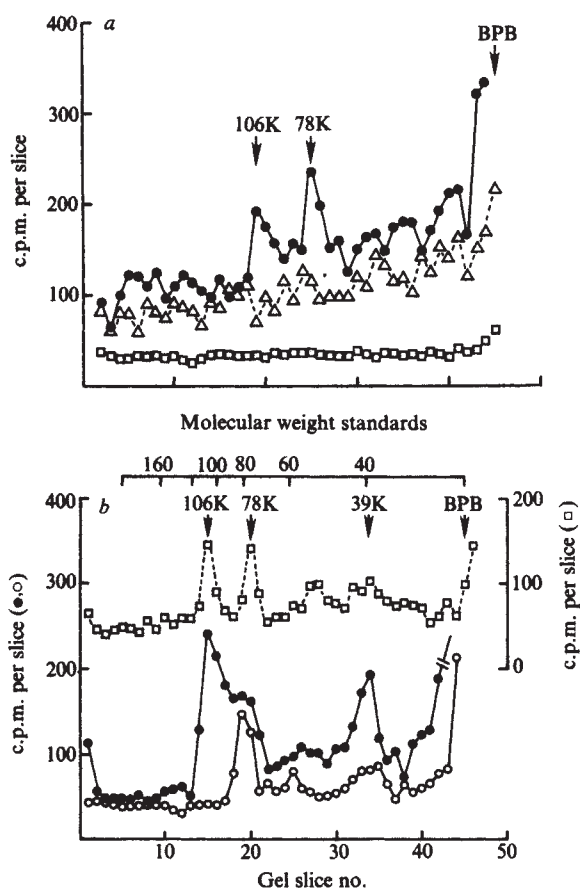


Fig. 1 Polyacrylamide gel electrophoresis of photolysed progesterone receptor preparations in denaturing conditions in 1% SDS by the method of Laemmli¹⁷. Total acrylamide monomer content was 7.5% in the separation gel. Gels were run in borosilicate glass tubes 6 mm $\times$ 10 cm, with 0.5-cm stacking gels. Electrophoresis of samples (10–100 μ l containing 0.01% bromophenol blue tracking dye) was at 2.5 mA per gel until the dye front had travelled 10 cm. Gels were removed, sliced and minced using a Savant automated slicer and then counted for ^3H in Amersham-Searle ACS scintillation cocktail at 25% efficiency. *a*, Electrophoresis of three companion receptor samples after labelling, precipitation and UV-ray irradiation for 30 min as described in the text. Samples were labelled with ^3H -R5020 ($\bullet$), ^3H -R5020 plus a 100-fold molar excess of nonradioactive progesterone (Δ), or with ^3H -progesterone ($\square$). Arrows identify two major labelled species whose labelling was time and irradiation dependent. *b*, Molecular weight determination of the major photoaffinity-labelled ^3H -R5020–protein complexes. A standard MW curve was determined on companion gels as shown by the upper abscissa. Standards were 5 μ g each of *Escherichia coli* RNA polymerase (165, 155, 95 and 39K bands), chicken conalbumin (86K), bovine serum albumin (68K) and chymotrypsinogen (26K). Protein bands were visualised by staining with Coomassie brilliant blue. Photolysed receptor– ^3H -R5020 complexes were prepared by 60 min UV-ray irradiation followed by dialysis against buffer A and lyophilisation. The samples were then redissolved in 20% of the original volume of sample buffer containing 1% SDS, and subjected to electrophoresis as in *a*. Unfractionated receptors ($\square$) were run as above or aliquots were chromatographed by stepwise KCl elution from DEAE-cellulose as described in the text to resolve receptor A and B proteins before electrophoresis. Two KCl steps were used: 0.15 M to yield protein A ($\circ$), and 0.3 M KCl to yield protein B ($\bullet$).

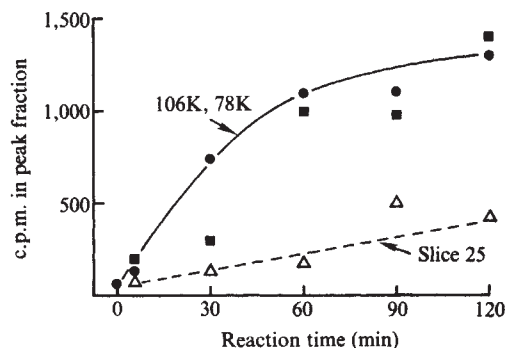


Fig. 2 Time course of photoaffinity labelling of progesterone receptors by ^3H -R5020. Chick oviduct cytosol was labelled for 120 min with 2×10^{-8} M ^3H -R5020 and then precipitated at 30% saturation of ammonium sulphate. The redissolved precipitate in buffer A was irradiated for the times indicated on the abscissa and then aliquots (1.0 ml) were frozen, lyophilised and subjected to SDS-polyacrylamide gel electrophoresis as described in Fig. 1 legend. The radioactivity in gel slices 13 ($\bullet$), 18 ($\blacksquare$) and 25 (Δ) are shown. These were the peak fractions corresponding to the labelled proteins of MWs 106,000, 78,000 and 39,000 respectively.

complexed with ^3H -R5020, ^3H -R5020 plus a 100-fold molar excess nonradioactive progesterone, or ^3H -progesterone alone. Saturation studies not shown here indicated that this amount of ^3H -R5020 was sufficient to occupy about 20% of the available receptor sites. At this ratio, negligible binding of ligand to non-receptor protein is detectable by competition assays. Figure 1*a* shows that the ^3H -R5020 was coupled to two major species, as indicated by the arrows. This labelling was eliminated by the presence of nonradioactive progesterone, whereas general labelling of the rest of the gel was not inhibited by the competitor. Labelled progesterone did not couple significantly to proteins, as expected, at a wavelength of >300 nm, which is the cutoff wavelength of the borosilicate glass filter. Figure 1*a* also shows considerable radioactivity running near the dye front; this material runs several fractions ahead of the front and probably represents ^3H -R5020 derivatives bearing charged groups produced by photolysis.

The extent of labelling seen on the gels was low, but consistent with the input noncovalent binding of ^3H -R5020 to receptor. For 150,000 c.p.m. ml^{-1} bound (by saturation analysis) and 10% efficient photoaffinity labelling (by TCA precipitation), the radioactivity which was recovered on the gels was about 50% of the theoretical value. This is a reasonably acceptable level when associated losses during transfer, dialysis and electrophoresis are considered.

We had shown previously¹⁸ that the two progesterone-binding receptor proteins B and A had molecular weights (MWs) of about 115,000 and 79,000. Thus we determined the MWs of the two covalent ^3H -R5020 peaks by comparison with electrophoresis of protein standards. The results are shown in Fig. 1*b*. Again, two distinct radioactive peaks were seen with equivalent radioactivity in each. Their MWs were 106,000 and 78,000 in this experiment. A protein band in the preparation, migrating at 39,000 g mol^{-1} was also labelled significantly, as shown by the arrow at 39K. These results provided additional evidence that the labelled proteins were, in fact, the desired receptor subunits.

As a third test, a photolysed ^3H -R5020–receptor preparation was made and fractionated by DEAE-cellulose chromatography on a 3.0-ml column equilibrated in buffer A as described previously⁴. Stepwise KCl elution at 0.15 M specifically elutes receptor A purified about 80-fold over cytosol; subsequent elution at 0.3 M KCl elutes receptor B, purified about 200-fold over cytosol but contaminated with about 30% receptor A. Samples from 5-ml reactions were chromatographed, desalted by dialysis in buffer A and subjected to SDS-polyacrylamide gel electrophoresis. As shown in Fig. 1*b*, 0.15 M KCl elution of ^3H -labelled receptor A yielded the 78,000 g mol^{-1} peak on the SDS gel; 0.3 M KCl elution yielded a major species at

106,000 g mol⁻¹. In addition, a shoulder occurred at 78,000 together with an augmented peak at 39,000. Thus, the DEAE step had resolved the two higher MW protein bands as predicted from the chromatographic behaviour of receptors A and B proteins.

To test the saturability and extent of the photoaffinity reaction to these proteins, a time course study of UV-ray irradiation was carried out using an excess of ³H-R5020 as the label, and aliquots (1.0 ml) were removed at various times. The samples were run individually on SDS-polyacrylamide gels by the method in Fig. 1; the peak fraction radioactivity of MW 106,000 and 78,000 were then plotted as a function of irradiation time. The results are shown in Fig. 2. The experiment shows a time-dependent covalent attachment of the label to the two receptor peaks, with a plateau at 1–2 h. Radioactivity in the peaks was now over 1,000 c.p.m., consistent with the use of additional input ligand. Significantly, both peaks became labelled at equivalent rates.

At least one additional peak of radioactive protein had been seen on the gels in Fig. 1, having a MW of about 39,000. It was possible that this was an additional receptor form, perhaps a proteolytic degradation product produced by an endogenous protease¹⁹. A hormone-binding intermediate in the digestion of the receptors has an apparent native MW of about 43,000 (ref. 20). Alternatively, the 39,000 g mol⁻¹ peak could be simply a non-receptor protein which became labelled due to its weak affinity for the ³H-R5020. Three types of test have been done which indicate that the latter alternative is the correct explanation. First, as shown in Fig. 1, radioactivity bound to protein on the gel in the low MW region was not competitively inhibited by simultaneous addition of nonradioactive progesterone. Thus, this 39,000 band was not a pre-existing receptor form present in the extracts. Second, in data not presented here, gels stained for protein with Coomassie brilliant blue showed a prominent band of protein at 39,000 g mol⁻¹, whereas the two higher MW bands did not coincide with stainable protein bands in these crude preparations. Thus, a potential contaminating protein of the proper mobility exists in the extracts. Third, Fig. 2 shows that the labelling kinetics of this 39,000 band differed markedly from that of the two receptor peaks. We therefore consider that the 39,000 band is probably a major protein contaminant, and becomes nonspecifically labelled with ³H-R5020.

We conclude that the photolytic method adopted here covalently attaches steroid R5020 to both progesterone receptor proteins in yields of about 10%. Additional side reactions also occur, resulting in at least labelling of a 39,000 g mol⁻¹ protein which is not receptor. This side reaction may arise as a consequence of photoactivation of the steroid; in the activated π^* state the ligand may bind less well to the receptor binding site and thus be released to interact with other proteins in the solution. As the 39,000 band protein is present in vast excess over the receptors, the efficiency of receptor–adduct formation in the system is very great compared with side reactions.

These studies thus provide direct physical determination of the MWs of the progesterone receptor components and provide additional evidence for the existence of two dissimilar hormone-binding subunits. As the ligand attachment is covalent, these labelled complexes should be ideal substrates for probing the hormone-binding site and as reagents in receptor radio-immunoassay. The receptor–labelling method should be generally applicable to other progestin receptors, including, for example, those of interest in breast tumours. Irradiation of tumour cells containing ³H-R5020 in tissue culture may also be a practical method for studying the ultimate fate of nuclear receptor–hormone complexes.

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Complete nucleotide sequence of immunoglobulin γ 2b chain gene cloned from newborn mouse DNA

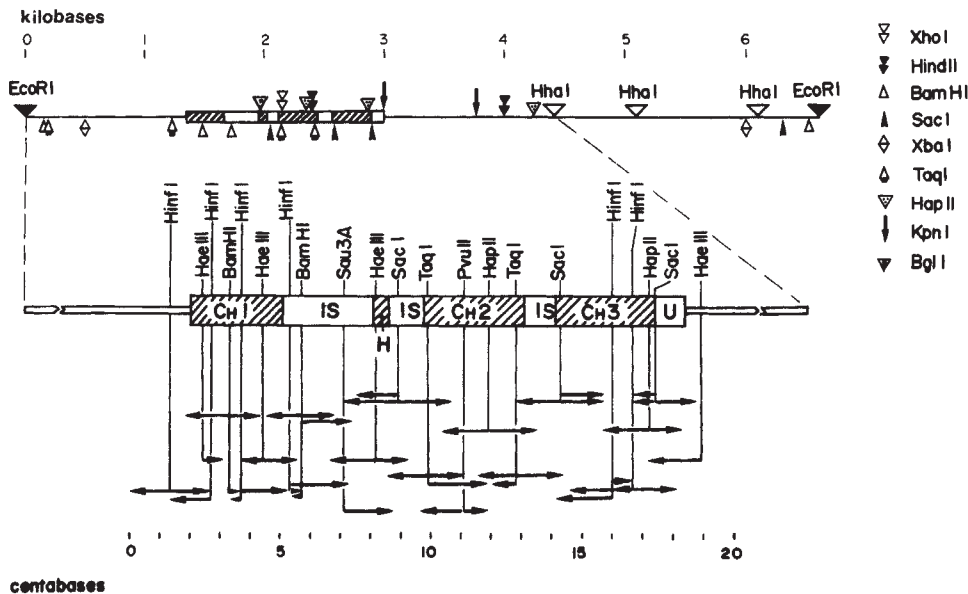
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Immunoglobulin heavy chain protein comprises five classes μ , γ , α , δ and ϵ chains which differ in amino acid sequences of the constant (C) region. The γ heavy chain class, which contains three domains and the hinge region, is divided into several subclasses such as γ 1, γ 2a, γ 2b and γ 3 in mouse. Amino acid sequence studies of mouse and human heavy chain proteins have shown that the γ subclass heavy chains are more closely related to each other than to the heavy chains of other classes. A similar conclusion was drawn from a nucleic acid hybridisation study using cDNAs complementary to the purified heavy chain mRNAs of the γ subclasses¹. These results suggest that the immunoglobulin heavy chain genes of the γ subclasses have evolved by relatively recent gene duplication of the ancestral γ chain gene. The complete nucleotide sequence of the γ 1 chain gene has demonstrated unequivocally that the domains and the hinge region are encoded in separate segments of DNA². Partial nucleotide sequences of the γ 2b chain gene indicated that the γ 2b gene is interrupted by intervening sequences (IS) at locations homologous to those in the γ 1 chain gene³. Comparison of the structures of these closely related genes would give new insights into evolution of eukaryotic genes and serve for understanding biological significance of various segments of the genes. We have now determined the complete nucleotide sequence of the immunoglobulin γ 2b chain gene cloned from newborn mouse DNA. The sequence not only predicts the complete amino acid sequence of the γ 2b chain but also demonstrates that the γ 2b chain gene is interrupted by three intervening sequences at the junction of the domains and the hinge region.

The 6.6-kilobase *EcoRI* fragment coding for the C region of the immunoglobulin γ 2b chain was previously cloned from newborn mouse DNA using λ gtWES-AB as an EK2 vector³. The recombinant phage was referred to as λ gtWES-IgH22 (abbreviated IgH22). The 6.6-kilobase fragment was isolated by agarose gel electrophoresis and ligated with the *EcoRI*-digested pBR322⁴. The hybrid plasmid was referred to as pBR322-IgH22 (abbreviated pIgH22). The detailed restriction

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relative to the mRNA sequence. The horizontal arrows shown at the bottom indicate the direction and the extent of the sequence determined. The restriction map of the cloned 6.6-kilobase *EcoRI* fragment is presented on the top line. The restriction sites which were used for sequencing in the 4.4-kilobase *EcoRI*-*HhaI* fragment are shown on the rectangle below. The $\gamma 2b$ chain gene is represented by the wide rectangle. CH_1 , CH_2 , CH_3 , domains of constant region; H, hinge region; IS, intervening sequence; U, untranslated sequence.

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20      40      60      80
0  GGAAATTTCTGACACTGCTTGCCTCAGATCAATTGTAGAAGACACGGTTCTAAGAGCAAGCTAAGAACAGATCTCTCCAAATATCCGAGGCCACTGAT
100 AAGAAAAAGCTCACACATTCTCTCTCTTGCAGCAAAACACACCCCATCAGTCTATCCACTGGCCCTGGGTGTGAGATACAACTGGTCTCTCCGT
200 T L G C L V K G Y F P E S V T V T W N S G S L S S S V H T F P A L
   GACTCTGGGATGCTGGTCAAGGGTACTTCCCTGAGTCAGTGTGACTTGAAGCTCTGGATCCCTGTCCAGCAGTGTGCACACTCTCCGAGCTCTC
300 L Q S G L Y T M S S S V T V P S S T W P S Q T V T C S V A H P A S
   CTGCAGTCTGGAGCTACACATGAGCAGCTCAGTGTGCTCCCTCCAGCACTTGGCCAGTCAAGCCTGACGCTGTGCTCACCAGCCAGGCT
400 S T T V D K K L
   GCACACGCTGGAGCAAACTTGGTGAAGAGCATTGAGGAGGAGGATTCACGAGAGTGGAGCAAGGTATAGCTATCTAAACAGCCAGGCT
500 GGGATCCATCACCAGGAGGTGACCTTAGCCAGGGAAGGAGGAGTACTGTCTGCTCCCTCTGGGAACATAGTATGACCACTACACTCAAG
600 GACATGTTCTCTGGGATAGGTGTGCTTGTTCATTTCAGGATCATCTGGAAGTAAAGCCATACAGGAGCAAACTTCTCTCTCTGTTTGGTGTCTCT
700 CTCCTTCAAAACAGTAACATCCAGCTTCTCTGTCAGAGCCAGCGGGCCATTTCAACATCAACCCCTGTCTCTCATGCAAGGAGTGTACAAAT
   C P
   C P
800 GCCCAGGTAAGTCACTACAGAGCTCCACTCCAGGAGAAATGGTAAAGTCTGTAATAAATCCCTGTAATGGAGGAAGCCATGACAAATCCATTCCAT
   A P N L E G G P L V F I F P P N I K D V L M I S L T P K V
   A P N L E G G P S V F I F P P N I K D V L M I S L T P K V
900 CTCTCTCATCAGCTCTACCTCGAGGGTGGAGCATCGGTCTTCACTTCCCTCAAAATCAAGGATGACTCATGATCTCCTGACACCAAGGTC
   T C V V V D V W E D D P D V Q I F V
   T C V V V D V S E D D P D V Q I S W F V N N V E V H T A Q T O T H R
1000 CGTGTGTGGTGGTGTGAGCGAGGATGACCCAGAGCTGCAGATCAGTGGTGTGTAACACAGCTGGAGTACACAGCTCAGACACAAACCCATAG
   S G K E F K C K V N N K
   E D Y N S T I R V V S L T C L P I O H O D W M S G K E F K C K V N N K
1100 AGAGGATTACAAGTACTACTCCGGGTGGTCAAGCTCCCATCCAGCAGGAGTGGATGAGTGGCAAGGATCAATGCAAGGTCAACAGCAA
   D L P P I E T I K I
   D L P S P I E R T I S K I K
1200 GACCTCCATCACCATCAGAGAGAACCTCTCAAAATTAAGGTGGGACCTGCAGGAGCACTGCATGGGGCTGGGATGGGCATAAGAAATAATGTCTA
   V
   G L V R A P O V V Y I L P P P A
1300 TGTGGACAGCTTCCACTTCAGCCATGACCTCTATGTATGTTTCTAATCCCAAGGGCTAGTCAGAGCTCCACAAGTATACATCTTGGCCACAGCAG
   E O L S R K D V S L T C L V V G F N P G D I S V E W T S N G H T E E
1400 AGCAGTGTGTCAGGAAAGATGTAGTCTCACTTGGCTGGTGGGCTTCAACCTGGAGACATCAGTGTGAGTGGAGCAGCAATGGGATCAGAGGA
   K T S K W E K T D S
   N Y K D T A P V L D S D G S Y F I Y S K L N M K T S K W E K T D S
1500 GAACACAGGACACCGACCATCTGAGGGTCTTACTCTCATATAGCAAGCTCAATAGAAACAGCAAGTGGGAGAAACAGATTC
   F S C N V R H E G L K N Y Y L K K T I S R S P G
   F S C N V R H E G L K N Y Y L K K T I S R S P G K T e r m
1600 TTCTCATGCAACGTGAGACAGAGGGTGTGAAAAATACTACCTGAAGAGACATCTCCGGTCTCCGGGTAAATGAGCTCAGCACCACAAAGCTCTC
   *poly(A)
1700 AGGTCTAAGAGACTGGCACCATATCCATGCATCCCTGTATATAAAGATCCAGCAAGGCTGGTACCATGTAAACTGTCTGGTCTTCTCCA
1800 AGGTATAGAGCATAGCTCAGGGCTGATGGGTCT

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Fig. 1 Sequencing strategy of the mouse $\gamma 2b$ chain constant region gene. Plasmid DNA pIgH22 was purified according to the method of Clewell and Helinski¹⁶. Restriction cleavage sites were determined by combined cleavages of two or more enzymes. *Bam*HI, *Sac*I, *Xho*I and *Kpn*I sites were determined previously³. The 4.4-kilobase *Eco*RI-*Hha*I fragment was isolated by 4% polyacrylamide gel electrophoresis after digestion of pIgH22 with *Eco*RI and *Hha*I, cleaved with other restriction enzymes and used as the substrates of phosphorylation. Alternatively, IgH22 was digested with either *Bam*HI or *Sac*I and resulting fragments were isolated. Each fragment was digested with the second restriction enzymes and used for phosphorylation. Sources of restriction enzymes were described previously². The length of sequenced fragment is given in centabases in the 5' to 3' direction

Fig. 2 Nucleotide sequence of mouse $\gamma 2b$ chain gene. The nucleotide sequence of the anti-coding strand is displayed 5' to 3'. DNA sequencing was performed according to the method of Maxam and Gilbert⁵. DNA fragments to be sequenced were digested with the enzymes indicated in Fig. 1, treated with bacterial alkaline phosphatase (Boehringer) and phosphorylated using T4 polynucleotide kinase (New England Biolabs) and [γ -³²P]ATP (NEN). ³²P-DNA fragments were isolated by polyacrylamide gel electrophoresis and cleaved with second restriction enzymes. Fragments labelled only at one end were isolated by polyacrylamide gel electrophoresis and purified as described previously⁴. For strand separation the labelled DNA samples were dissolved in 100 μ l of 0.2 M KOH, 10% (w/v) glycerol, 0.01% (w/v) xylene cyanol and 0.01% (w/v) bromophenol blue, heated at 37°C for 1 min and separated with 15% or 20% polyacrylamide gel. Four base-specific reactions were utilised (G, A > C, C + T, C). Samples were loaded in 90% formamide and separated with thin gels (0.5 $\times$ 300 $\times$ 400 mm, 8 and 20% polyacrylamide gel). The amino acids predicted by the nucleotide sequence are shown above the coding sequences in italics. The partial amino acid sequences of MOPC 141⁶ and MPC 11⁷ are presented on the top line. Term, termination codon; poly (A), putative poly (A) addition site assigned by comparison with the mouse $\gamma 1$ chain gene sequence. Amino acids are expressed by one letter code of A(Ala), C(Cys), D(Asp), E(Glu), F(Phe), G(Gly), H(His), I(Ile), K(Lys), L(Leu), M(Met), N(Asn), P(Pro), Q(Gln), R(Arg), S(Ser), T(Thr), V(Val), W(Trp) and Y(Tyr).



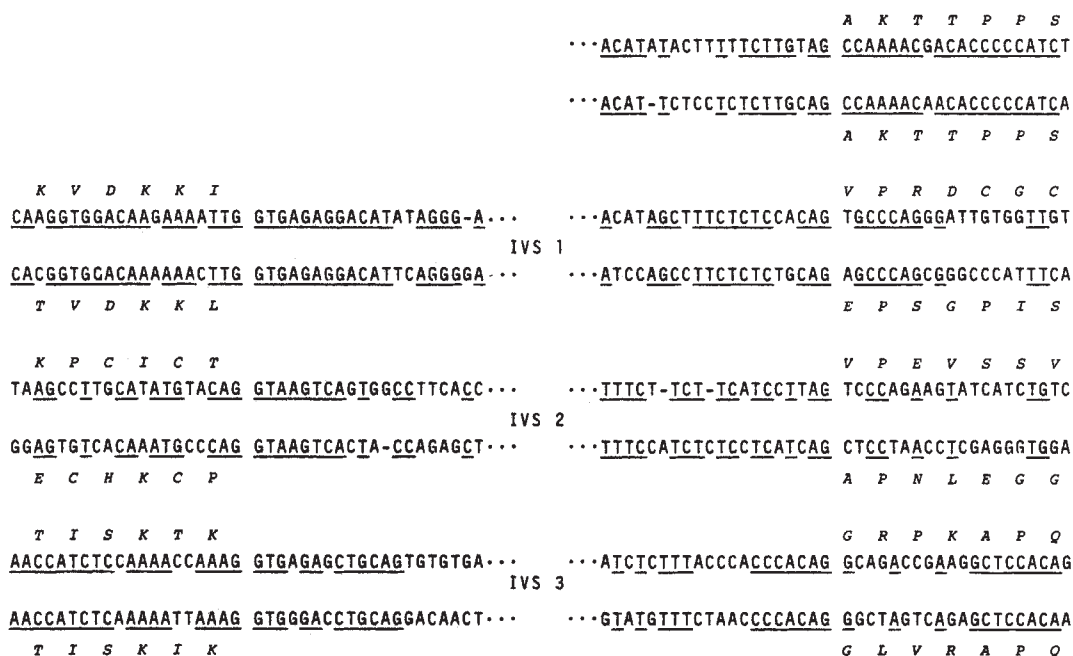
Fig. 3 Comparison of the amino acid sequences of $\gamma 1$, $\gamma 2a$ and $\gamma 2b$ chains. The amino acid sequences of the $\gamma 1$ and $\gamma 2b$ chains were predicted by the nucleotide sequences of the respective genes cloned from newborn mouse DNA². The amino acid sequence of the $\gamma 2a$ chain was taken from the data of MOPC 173^{7,8}. The residue was numbered with the first residue of C_{H1} domain as 118. Homologous positions are boxed. The one letter code used is as described in Fig. 2.

enzyme cleavage map of the *EcoRI*–*HhaI* fragment was constructed and the nucleotide sequence was determined by the chemical modification method of Maxam and Gilbert⁵. The restriction enzyme cleavage sites used for sequencing and the direction and range of sequencing are shown in Fig. 1.

The complete nucleotide sequence of the $\gamma 2b$ chain C region gene is shown in Fig. 2. The total number of the nucleotides determined is 1,834, which include the coding sequence, the IS, the 3' untranslated sequence and the 5' and 3' flanking sequences.

The nucleotide sequence predicts the complete amino acid sequence of the C region of the $\gamma 2b$ chain (336 residues) which consists of the C_{H1} (residue 118–214), C_{H2} (residue 237–346) and C_{H3} domains (residue 347–453) and the hinge region (residue 215–236), respectively. The predicted sequence was compared with the partial amino acid sequences so far published, including 18 residues in the hinge region and the C_{H2} domain⁶, 66 residues in the C_{H2} domain and 35 residues in the C_{H3} domain⁷ (Fig. 2). The amino acid sequences derived from the nucleotide sequences matched the known amino acid

Fig. 4 Comparison of nucleotide sequences of the $\gamma 1$ and $\gamma 2b$ chain genes around the junction of IS and coding sequences. Twenty nucleotides each of the homologous IS and coding sequences of the $\gamma 1$ (upper sequences) and $\gamma 2b$ (lower sequences) chain genes are aligned to maximise homology. The top row is the 5' end of the C_{H1} domain. The second, third and fourth rows are the 5' and 3' ends of the first, second and third IS, respectively. The nucleotide sequence of the strand corresponding to the mRNA is displayed 5' to 3' direction. The homologous nucleotides are underlined. Amino acids predicted from the nucleotide sequences are shown in italic letter by one letter code as described in Fig. 2.



sequences except two residues, Ser in place of Leu at residue 240 and Ser in place of Tyr at residue 268. To determine the junctions of the IS and coding sequences (see below) the nucleotide sequences were also correlated with the amino acid sequences of the $\gamma 2a$ chain^{7,8} which share extensive homology with the known amino acid sequences of the $\gamma 2b$ chain⁷.

The complete amino acid sequence of the C region of the $\gamma 2b$ chain is compared with the amino acid sequences of the $\gamma 1^2$ and $\gamma 2a^{7,8}$ chains as shown in Fig. 3. It is evident that the $\gamma 2b$ and $\gamma 2a$ chains show more homology to each other than to the $\gamma 1$ chain. The results agree with our previous conclusion from cDNA-mRNA hybridisation studies¹. The homology between the three γ chain subclasses ($\gamma 1$, $\gamma 2a$ and $\gamma 2b$) for each domain is calculated. The C_{H1} domains are most conserved (79–82%) throughout the three subclasses while the C_{H3} domains are least conserved (53–59%). The C_{H2} domains are highly conserved between the $\gamma 2a$ and $\gamma 2b$ chains whereas they are moderately conserved (63–68%) between the $\gamma 1$ and $\gamma 2b$ (or $\gamma 2a$) chains. The G + C content of the coding sequences is 52.3%, exceeding the value (41.8%) of the total mouse DNA⁹.

Note that the nucleotide sequences predict an unexpected Lys residue at the carboxy terminus of the $\gamma 2b$ chain. Although most of the γ chains so far sequenced end with the Pro-Gly residues¹⁰, the nucleotide sequences of the cDNA as well as chromosomal gene of the $\gamma 1$ chain also predict an additional Lys residue at the end of the heavy chain^{2,11}. These results suggest that the newly-synthesised $\gamma 2b$ chain proteins may have a Lys residue at the carboxy terminus, which will be removed eventually either at secretion or during circulation in the blood. It would be interesting to see whether the $\gamma 1$ and $\gamma 2b$ chain proteins bound to the cellular surface have the Lys residue at their carboxy terminus since the amino acid sequencing was done using secreted immunoglobulins. The membrane-bound and secreted μ chains are reported to be different in the primary structures of their carboxy termini^{12,13}.

The comparison of the nucleotide sequences of the $\gamma 2b$ chain gene with the amino acid sequences of the $\gamma 2b$ and $\gamma 2a$ chains indicates that the domains and the hinge regions of the $\gamma 2b$ chain structural sequence are separated by three ISs as shown in Figs 1 and 2. The ISs split the $\gamma 2b$ chain gene at positions homologous to those in the $\gamma 1$ chain gene^{2,14}. The first, second and third ISs of the $\gamma 1$ and $\gamma 2b$ chain genes as a pair are 356/316, 98/107, 120/112 base pairs long, respectively. The evolutionary significance of the ISs in immunoglobulin genes was discussed more extensively in previous reports^{2,3}.

The boundary sequences are compared between the $\gamma 1$ and $\gamma 2b$ chain genes (Fig. 4). The first 10 nucleotides at each end of the ISs are highly conserved, although not identical, between the two genes whereas there is considerable divergence of the overall nucleotide sequences of the homologous IS. The possibility is discussed that not only nucleotide sequences at the joints of the IS and structural sequences but also other signals such as secondary and tertiary structures of mRNA precursors may be important for the recognition of splicing¹⁵.

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A novel fragment of the corticotropin- β -lipotropin precursor

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The pituitary ACTH/MSH cells are virtual storehouses for biologically important peptides. In addition to corticotropins, melanotropins, opioid peptides and lipolytic peptides these cells contain large amounts of at least three different polypeptides (or small proteins) having tryptophan in the amino-terminal position (Trp-peptides)^{1–3}; their approximate molecular weight (MW) has been given as 14,000 (ref. 4). Nothing is known of their chemical properties and physiological significance. Experimental data indicate that the Trp-peptides in the ACTH cells are stored in the secretory granules³ to be released jointly with ACTH and other secretory products, such as β -endorphin and/or β -lipotropin (β -LPH)⁶. ACTH and β -LPH are formed from a large common precursor protein where they constitute the carboxy-terminal portion^{7–14}. Together the two peptides account for one-third to one-half of the molecular weight of the precursor and there has been considerable interest in the primary structure and possible physiological significance of the remaining amino-terminal portion¹⁵. The fact that the Trp-peptides occur in conspicuously high concentrations in the secretory granules of the ACTH/MSH cells prompted an investigation into their relation to the ACTH- β -LPH precursor protein. We have now isolated a glycopeptide with an apparent MW of ~11,000 residing in the secretory granules of the ACTH/MSH cells from pig pituitary. The amino acid sequence of its amino-terminal part was found to be identical with the amino-terminal end of the bovine corticotropin- β -LPH precursor protein¹⁶. We propose that the isolated new fragment is identical with the amino-terminal portion of the precursor that remains after ACTH and β -lipotropin have been split off.

Acetone powder was prepared from fresh frozen pig pituitaries and extracted with hot saline. The pH of the extract was adjusted to 3.9, the resulting precipitate was spun down and the supernatant was dialysed and freeze-dried (Table 1). The freeze-dried material was chromatographed on a DEAE-Sephadex column. The Trp-peptides could be separated into several peaks, the three major ones were labelled I, II and III (Fig. 1a). Each of the three Trp-peptides were further purified by gel chromatography (Fig. 1b). As a result, all of them appeared highly purified but only peptide I satisfied the criteria for complete homogeneity as judged by SDS-gel electrophoresis (Fig. 1c) and amino-terminal analysis. Tryptophan was identified as the amino-terminal amino acid (see below).

An antiserum was raised against peptide I. In a radioimmunoassay system this antiserum cross-reacted weakly with β -LPH and not at all with ACTH, α -MSH, β -MSH, β -endorphin or β -LPH_{39–45}. The antiserum was used to localise peptide I in the pituitary by immunohistochemistry. The results confirmed our previous fluorescence histochemical finding³ that the Trp-peptide resides in the secretory granules of the ACTH/MSH cells (Fig. 2).

Peptide I was not recognised by antisera either to ACTH_{1–24} (ACTH radioimmunoassay kit, Amersham Radiochemical Centre) or to α -MSH (ACTH_{1–13}) which suggests that neither ACTH nor α -MSH is contained within its structure.

The amino acid sequence of the 1–35 portion of the amino-terminal end of peptide I was: Trp-Cys-Leu-Asp-Ser-Ser-Gln-Cys-Gln-Asp-Leu-Ser-Thr-Glu-(?) -Asn-Leu-Leu-Ala-Cys-Ile-Arg-Ala-Cys-(?) -Pro-Asp-Leu-Ser-Ala-Glu-Thr-Pro-Val-Phe. Comparison of the identified amino acid sequence with that

of previously identified peptides and proteins showed it to correspond almost exactly to that recently predicted by Nakanishi *et al.*¹⁶ for the amino acid sequence -105 to -71 of the ACTH- β -LPH precursor (Fig. 3). The prediction was based on nucleotide sequence analysis of cloned cDNA encoded by mRNA from bovine pituitary intermediate lobe. Our results differed from the prediction in position -102 where we found Asp instead of Glu, and in position -94 where we found Ser instead of Thr. These differences may be ascribed to the fact that the predicted amino acid sequence was based on a bovine nucleotide sequence whereas we have used porcine material. Since immunochemical analysis indicated that neither ACTH nor α -MSH are contained within the structure of peptide I and since the prediction has readily hydrolysable Lys-Arg in position -2 and -1 connecting with ACTH (ref. 17), it appears likely that the carboxy terminus corresponds to position -3 in the predicted ACTH- β -LPH precursor (Fig. 3). The amino acid composition of peptide I is given in Table 2. The presence of

galactosamine and glucosamine residues suggests that peptide I is glycosylated. This finding is consistent with the observation that the ACTH- β -LPH precursor is a glycoprotein¹⁸.

The remarkable similarities in amino acid sequence between the amino-terminal portion of peptide I and a part of the predicted ACTH- β -LPH precursor make it highly probable that peptide I is a fragment of the ACTH- β -LPH precursor. In addition, we propose that the isolated Trp-peptide exists as such in the secretory granules of the ACTH/MSH cells. It seems unlikely that the peptide is generated by cleavage at the Trp position during the process of purification, because specific fluorescence histochemical analysis has revealed the presence within these cells of extremely large amounts of polypeptides (or small proteins) having tryptophan at the amino terminus¹⁻³. Thus, we believe that peptide I is a naturally occurring fragment of the ACTH- β -LPH precursor. The carboxy terminus of peptide I probably corresponds to position -3 in the ACTH- β -LPH precursor. If so, peptide I is the amino-terminal portion

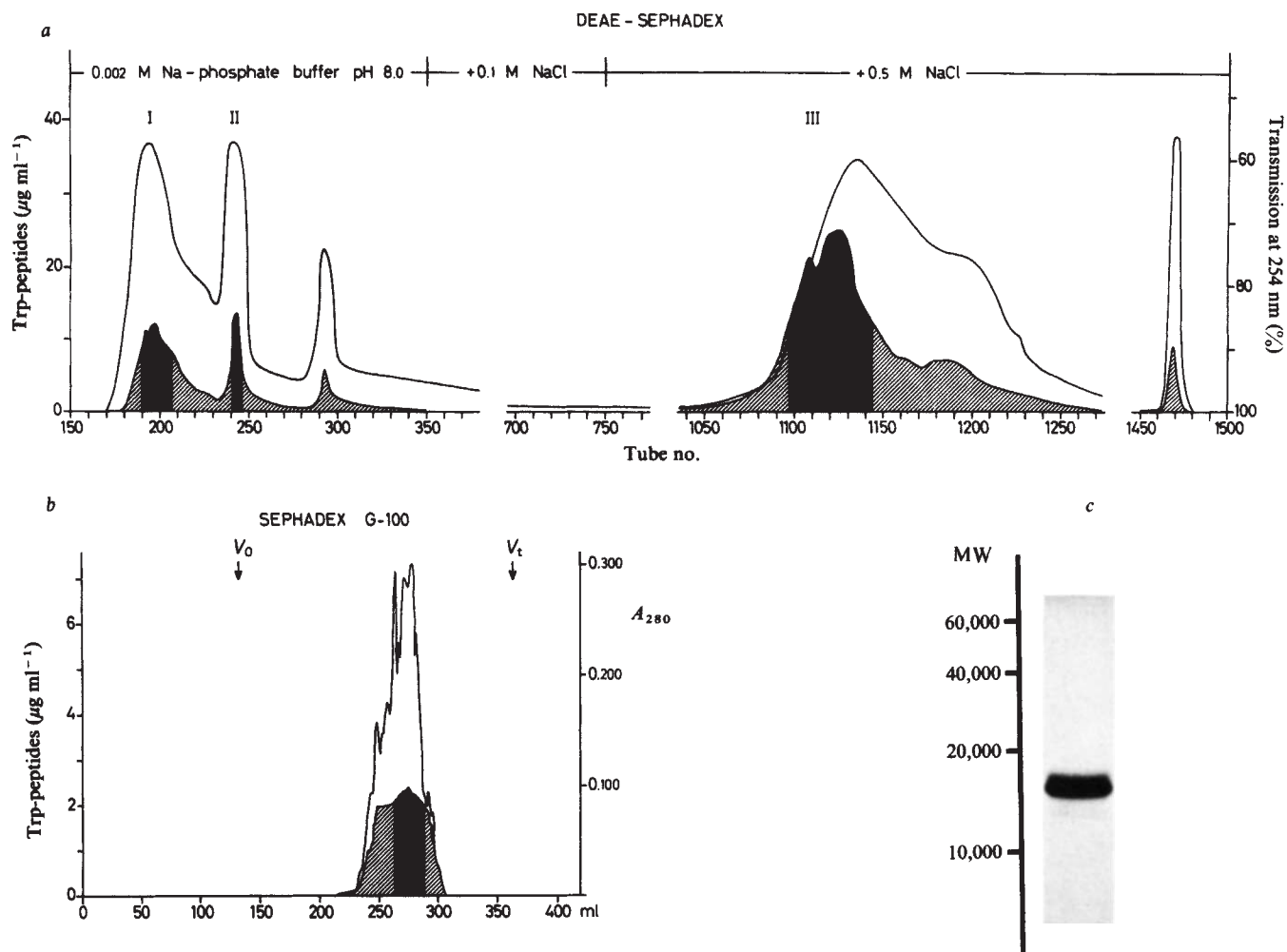


Fig. 1 *a*, Chromatography on a DEAE-Sephadex A-25 column (gel bed height 160 cm, diameter 5 cm) equilibrated with 0.002 M Na-phosphate buffer, pH 8.0. Freeze-dried extract of pig pituitaries (see Table 1) was dissolved in the buffer and applied to the column (3 g in 30 ml). The column was developed with the dilute phosphate buffer. Fractions of about 4 ml were collected. After the appearance of non-adsorbed proteins the elution buffer was enriched first with 0.1 M NaCl and then with 0.5 M NaCl. The stippled area shows the elution pattern of the Trp-peptides, measured fluorometrically²⁴. The material collected for further purification is indicated in black. Peaks I, II and III were dialysed against redistilled water for at least 48 h and freeze-dried. The lyophilised material in peak I weighed 135 mg and was purified 5.3-fold compared with the acetone powder in Table 1. It was taken up in 0.1 M formic acid to a concentration of 50 mg ml^{-1} and applied to a Sephadex G-100 superfine column (gel bed height 90 cm, diameter 2.5 cm) equilibrated with 0.1 M formic acid, which was also used for elution (*b*). Fractions of 4 ml were collected and analysed for Trp-peptides. V_0 is the void volume defined by the use of blue dextran. V_t is the total mobile phase defined by the use of $^3\text{H}_2\text{O}$. The weight of the purified material after dialysis and freeze-drying was 40 mg (Trp-peptide I). It was purified 6.2-fold. *c*, SDS-polyacrylamide (12.5%) gel electrophoresis of the isolated Trp-peptide (peptide I) was performed in a discontinuous system according to the method of King and Laemmli³⁰. Samples were pretreated with 0.1 M 2-mercaptoethanol and heated at 90°C in the presence of 1% SDS for 3 min. Separations were carried out in a Pharmacia apparatus GF-4 and proteins and peptides were stained with Coomassie brilliant blue R-250. The approximate molecular weight scale was deduced from the positions of nine standard proteins. The molecular weight of the isolated peptide appears to be over 15,000. This assessment, however, does not take into consideration the possibility that the peptide is rich in basic amino acids and/or glycosylated which may result in overestimation of the molecular weight.

Table 1 Recovery and yield of Trp-peptides in the initial purification steps

Preparation	Weight (g)	Protein (g)	Trp-peptides (mg)*	Calculated yield of Trp-peptides (%)	Relative purification† (-fold)
Acetone powder	225	140	150	100	1
Saline extraction (95–100 °C)	—	16	60	40	3.5
Precipitation at pH 3.9	13	10	50	33	4.7

One kg frozen pig pituitaries was homogenised in 10 l of cold acetone and left overnight. The resulting precipitate was washed with 40 l acetone and with 20 l acetone-diethyl ether (1:1) and finally with 20 l diethyl ether. The dried acetone powder was extracted with 4 l hot saline (95–100 °C) for 10 min. After cooling to 4 °C and centrifugation at low speed the pH of the supernatant was adjusted to 3.9 by the dropwise addition of 1 M HCl. After boiling for 3 min, the resulting precipitate was spun down and discarded, and the supernatant was dialysed against several changes of re-distilled water (at least 48 h) and freeze-dried. The concentration of Trp-peptides was determined fluorometrically with tryptophyl-alanine as standard²⁴. Values are means of four separate runs. Protein was measured according to the method of Lowry *et al.*²⁵.

* Peptide weight expressed in terms of weight-equivalents of tryptophyl-alanine.

† Purification calculated from the ratio of μg Trp-peptides over mg protein.

that remains after β -LPH, ACTH, and the connecting Lys-Arg pair have been split off¹⁷.

The prediction of the amino acid sequence of the ACTH- β -LPH precursor does not identify the amino terminus with certainty. Met in position -131 was considered a likely location for the translational initiation site¹⁶. We propose that the amino terminus of the precursor, following removal of the signal peptide, is Trp in position -105 (Fig. 3). It is probable that the amino acid sequence from -131 to -106 constitutes the signal peptide which is included in the primary translational product but lost upon packaging in the secretory granules. Hence, the

granule-stored amino-terminal fragment of the porcine ACTH- β -LPH precursor consists of 103 amino acid residues and has a molecular weight of approximately 11,000 (Table 2).

The ACTH- β -LPH precursor (239 amino acid residues, MW ~26,000) generates β -LPH, ACTH and peptide I through enzymatic hydrolysis of consecutive basic residues¹⁷. Based on the occurrence of such consecutive basic residues within the precursor molecule other points of cleavage can be postulated.

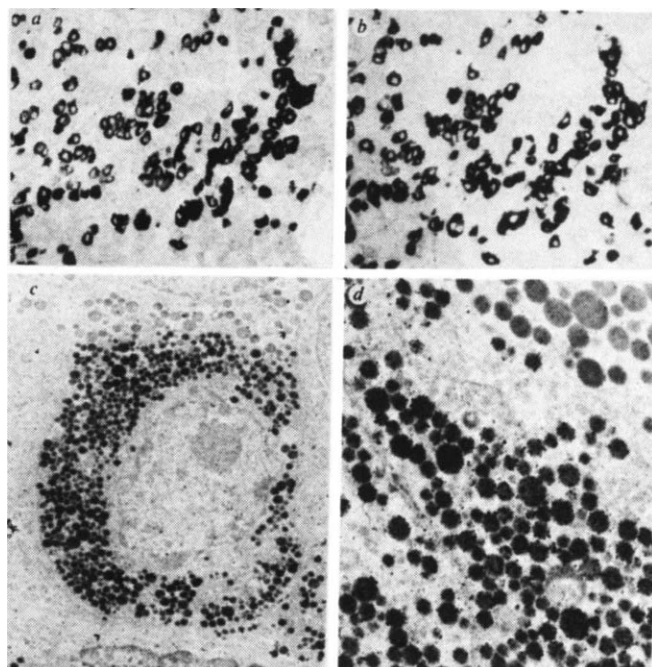


Fig. 2 Immunostaining of cells in the pig pituitary (anterior lobe) using antiserum to Trp-peptide I (a) and ACTH (ref. 3) (b). Three rabbits were immunised with peptide I by subcutaneous injection (multiple site) of the antigen (200 μg) emulsified with 1 ml of Freund's adjuvant. Booster injections were given 4 weeks later. Satisfactory antibody titre for radioimmunoassay (1:1,000) was obtained in one of the rabbits 2 weeks after the second injection. The cells displaying peptide I immunoreactivity were identical with the ACTH cells as shown by immunostaining consecutive plastic sections. Formaldehyde-fixed tissue was embedded in Epon. Sections, 1- μm thick, were exposed to either of the two antisera (each in dilution 1:320) for 24 h at 4 °C. The site of antigen-antibody reaction was identified by the peroxidase-antiperoxidase method of Sternberger³¹. The intracellular storage site of peptide I was established by electron immunocytochemistry (c, d). Note intense immunostaining over all secretory granules of the ACTH cell. Non-reacting cell (probably GH cell) in upper right corner of d. (a and b $\times 220$; c $\times 3,100$; d $\times 9,900$.)

ACTH may yield α -MSH (ACTH₁₋₁₃) and CLIP (ACTH₁₈₋₃₉). β -LPH yields γ -LPH (β -LPH₁₋₅₈) and β -endorphin (β -LPH₆₁₋₉₁). The amino-terminal part of the precursor (peptide I) may yield a smaller Trp-peptide (residues -103 to -58), γ -MSH (residues -56 to -43) and a carboxy-terminal fragment (residues -41 to -1) (Fig. 4).

The fact that the isolated amino-terminal part of the ACTH- β -LPH precursor exists as such in the secretory granules of the ACTH/MSH cells and that it is released together with other secretory products upon stimulation⁶ makes it tempting to speculate that it exerts a physiological function in the circulation. Some preliminary tests of bioactivity have been performed. The melanotropic activity, tested in a bioassay system¹⁹, was such that 1 μg of the Trp-peptide gave the same response as 3.4×10^{-3} μg α -MSH (Sigma). This probably reflects the His-Phe-Arg-Trp sequence (-51 to -48) which is known to carry melanotropic activity²⁰. The peptide had weak lipolytic activity on isolated rabbit adipocytes²¹; 1 μg of the peptide gave about the same response as 10^{-2} μg β -LPH. The peptide had no

Table 2 Amino acid composition of peptide 1

Residue	Residues determined*	Residues calculated to the nearest integer	Residues expected from predicted amino acid sequence -105 to -3
Lys	3.5	4	3
His	0.9	1	1
Arg	7.2	7	9
Asp	9.4	9	11
Thr	3.5	4	5
Ser	8.4	8	8
Glu	12.7	13	15
Pro	8.1	8	8
Gly	15.9	16	13
Ala	8.1	8	7
Half-cystine	6.6	7	4
Val	3.0	3	5
Met	1.1	1	1
Ile	1.4	1	1
Leu	7.1	7	6
Tyr	1.3	1	1
Phe	3.2	3	3
Trp†	1.5	2	2
Glucosamine	2.5	++	0
Galactosamine	0.6	+	0

Amino acid analyses were performed by hydrolysing samples of peptide I (100–200 μg) in 0.5 ml of boiling 5.7 M HCl at 110 °C for 24 and 72 h in sealed evacuated tubes. The hydrolysates were analysed according to the two column method of Spackman *et al.*²⁶ in a Durrum D-500 amino acid analyser. Carbohydrates were estimated on the hydrolysis samples. Half-cystine was determined as cysteic acid and methionine as methionine sulphone after performic acid oxidation²⁷. Tryptophan was determined after hydrolysis in methanesulphonic acid²⁸ and before hydrolysis by a spectrophotometric method²⁹.

* All values are given in residues per 103 residues total amino acids (values for amino sugars and ammonia not included in summation). 103 is the number of residues expected from the predicted amino acid sequence -105 to -3. The calculated molecular weight of the peptide sequence is approximately 11,000.

† With both methods of determination the number of tryptophan residues was between 1 and 2.

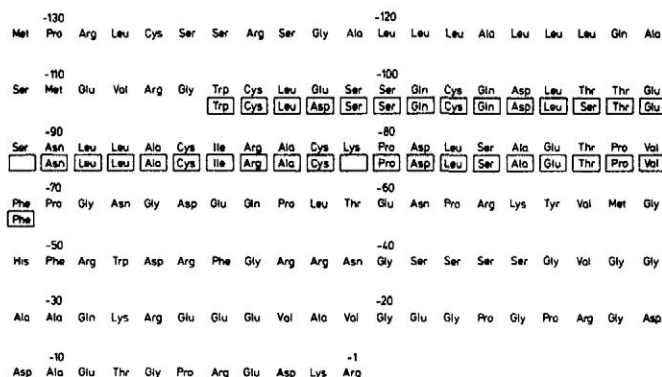


Fig. 3 The predicted amino acid sequence of the amino-terminal portion of the bovine ACTH- β -LPH precursor after ACTH and β -LPH have been split off (according to Nakanishi *et al.*¹⁶). The amino acid residues are numbered in the direction from amino terminus to carboxyl terminus, with the first residue proximal to ACTH given as no. -1; being on the NH₂-terminal side of ACTH all the residues are indicated by negative numbers (see ref. 16). Amino acid residues in boxes represent the identified primary structure of the Trp-peptide isolated from pig pituitary. Amino acid sequencing³² was made with a Beckman sequencer (model 890 C) using the standard Beckman 1 M Quadrol program (Beckman 122974) with polybrene to retain the peptide^{33,34}. Phenylthiohydantoin (PTH) derivatives were identified by high-pressure liquid chromatography on a 30-cm Waters μ -Bondapak C18 column by using stepwise elution with a system with methanol-containing buffers (Waters Associates) for the ethylacetate phases and a 60/40 mixture of methanol and 0.01 M sodium acetate for the aqueous phases. TLC on fluorescent silica gel plates was used as an additional method of identification³⁵. Moreover, PTH-carboxymethylcysteine residues were identified by radioactive labelling.

adrenocorticotrophic activity when tested *in vivo*²², and it did not cause displacement of naloxone in an opiate receptor-ligand assay²³. The functional significance of peptide I and of the other pituitary Trp-peptides (which may well be fragments of peptide I) remains to be established.

After submission of this paper, Keutmann *et al.*³⁶ described the partial purification from mouse pituitary tumor cells of a glycoprotein accounting for most of the non-ACTH, non- β -LPH region of the ACTH- β -LPH precursor. The amino terminus was found to be tryptophan.

The sequencing data are available from the authors on request. We thank Dr David Eaker for determining the amino

acid composition, Dr Leonard Larsson for performing the assay of melanotropic activity, Dr Per Melin (Ferring AB) for performing the assay of adrenocorticotrophic activity and Professor P. Schwandt for assaying the lipolytic activity. Antiserum against α -MSH was a gift from Dr D. Swaab. The investigation was supported by grants from the Swedish Medical Research Council (04X-1007) and from the Medical Faculty of Lund, Lund, Sweden.

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Recovery frequency of phages λ and M13 from human and animal faeces

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Derivatives of bacteriophages λ and M13 are in common use as vectors in recombinant DNA research¹⁻⁵. These laboratory-derived phages have been designed to allow cloning of DNA fragments, but to be unable to survive outside a defined laboratory and/or host-cell environment. To assess the availability of wild-type λ or M13 phages in the environment which might potentially rescue debilitated derivative phages, we have now examined the frequency of these and other bacteriophages in human and animal faeces. We detected coliphage in over two-thirds of the faecal samples. Of these, 1.2% of the samples contained λ -like phage and 3.5% had phage indistinguishable from M13.

Bacteriophage λ was originally discovered as a prophage in *Escherichia coli* K12 by Lederberg⁶. It has been the subject of extensive genetic and molecular studies. The Charon phages 3A, 4A and 16A are derivatives of λ which have been certified for recombinant DNA studies in an EK2 system⁷ when they are grown in debilitated *E. coli* K12 DP50 or DP50 *supF* (ref. 1).

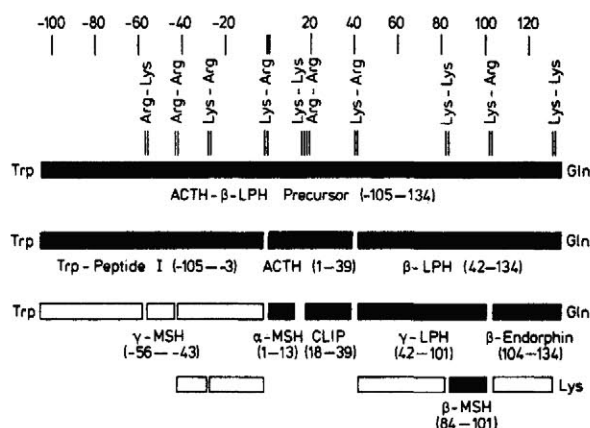


Fig. 4 The proposed ACTH- β -LPH precursor protein (putative signal peptide not included) and the various fragments that may be generated from the precursor by proteolytic processing, assuming a protease specific for consecutive basic residues¹⁷ acting on the many pairs of Arg or Lys (indicated in the figure) connecting the component peptides in the precursor molecule. Identified or postulated fragments are shown in black contrast to purely hypothetical fragments.

Another derivative of phage λ , λ -gt WES B (refs 2, 3), was produced by introducing amber mutations and other modifications into phage λ -gt- λ C (ref. 4). It has also been certified as an EK2 vector when grown in *E. coli* K12 DP50 supF. M13 is a male-specific DNA phage originally isolated by Hofschneider⁸ from a strain of *E. coli*. Derivatives of this phage have been produced for use as cloning vehicles⁵.

Table 1 *E. coli* strains studied

Strain	Relevant characteristics	Source
D0-27	<i>E. coli</i> CA ^s , F ⁻	M. Malmay
D0-28	<i>E. coli</i> CA ^s , F ⁻ nal ^R	This laboratory*
D0-29	<i>E. coli</i> CA ^R mal ⁻ F ⁻ nal ^R	This laboratory†
B0-11	<i>E. coli</i> CA ^R nal ^R F ⁺	This laboratory
U-7	<i>E. coli</i> K12 HFr (λ ⁺)	M. Malmay

* Nalidixic acid resistant mutants of D0-27 were isolated without mutagenesis after heavy inoculate plating on MacConkey agar containing 30 μ g ml⁻¹ nalidixic acid.

† A spontaneous, λ resistant mal⁻ derivative of D0-28. Resistance to λ was confirmed by inability of λ ^{vir} to grow.

In our study, four populations of humans were sampled—hospital patients, personnel from clinical and research laboratories, first-year medical students living in the Boston area (urban), and people residing in Sherborn, Massachusetts (rural). Animals from a farm in Sherborn, 20 miles west of Boston, were also sampled. Faecal specimens were collected on sterile 'Cultures' (Scientific Products) and streaked onto MacConkey plates. Phage assays were carried out as described in Table 2 using strains listed in Table 1. During the initial screening process, the presence of phage λ was detected by identifying those plaques on strain D0-28 (*E. coli* CA^s, F⁻) which did not appear on strain D0-29 (*E. coli* CA^R, F⁻). The presence of M13 was detected by its ability to form plaques on strain B0-11 (*E. coli* C, F⁺), but not on D0-29.

Among the 303 samples tested in both studies, phage were detected at an average frequency of 69% (Table 2). The highest percentage of samples with phage was found in the urban and laboratory groups. This finding agrees with a recent report⁹ that urban sewage yielded a higher concentration of phages than

rural sewage. Phage isolates suspected of being λ were tested for DNA homology with known λ phage using DNA-DNA filter hybridisation¹⁰ to ³²P-labelled λ 55 (ref. 11). In addition, the plaque isolates were tested for their ability productively to infect λ -lysogenic *E. coli*. Homimmune λ -like phages should produce no plaques on λ -lysogenic *E. coli* due to the immunity conferred on lysogenic cells by the λ repressor. By the above criteria, only 2 of the 162 samples (frequency 1.2%) contained bacteriophage which were indistinguishable from λ . They came from a rural housewife and one of the laboratory personnel.

The M13 isolates formed turbid plaques on the F⁺ indicator strain (B0-11), but no plaques on the F⁻ strain (D0-28). To confirm that these isolates were M13, each was hybridised¹⁰ to a ³²P-labelled RF M13. This probe was made by nick translation¹² of RF DNA. Additional confirmation that the plaques were M13 came from agarose gel electrophoresis of supernatants from phage-infected cells in which the DNA was easily detected by ethidium bromide staining of the gels (L. Laverne, J. Hines and D. Ray, personal communication). Each M13 isolate collected from a productive infection of B0-11 showed a mobility identical to native M13 (Fig. 1). Furthermore, suspected M13 phages were neutralised by antiserum directed against the M13 coat protein.

M13-like phages were recovered in 5 out of the 141 samples (3.5%) from humans. The highest frequency was found in hospital (9.1%) and rural (farm) (5.9%) samples. M13 was detected at the same frequency (5.9%) in samples from farm mammals, but was not recovered in chicken or fly samples from the same farm.

Our findings provide the first data regarding frequency of λ and M13 in man's immediate environment. As these phages naturally infect *E. coli*, their presence would be most evident in faecal samples. In this series of tests, both phages were easily found, but at relatively low frequencies. Our screening method identified wild-type phages with receptors (tail fibres) similar to λ or M13. However, it would not have detected M13-like and λ -like phages having regions of DNA homology and potential rescue ability, but different receptors. Thus heteroimmune lambdoid phages, such as 21, 82, 424 and 434 (ref. 13), would have been detected, but not ϕ 80 (ref. 14) which has a different receptor.

The frequency of M13 has not previously been assayed. Dhillon *et al.*⁹, assaying Hong Kong sewage, found 24 male-

Table 2 Frequency of phage isolation from faecal samples in different environments

Human samples	No. of samples tested for λ	% Samples with any phage	% Samples with λ -like phage	No. of samples tested for M13	% Samples with any phage	% Samples with M13
Hospital	50	44.0	0	33	60.6	9.1
Laboratory	39	84.6	2.6	48	75.0	0
Urban	48	89.6	0	43	72.1	2.3
Rural	25	68.0	4.0	17	41.2	5.9
Total	162	71.0	1.2	141	66.7	3.5
Non-human samples						
Flies	4	75.0	0	7	28.6	0
Chickens	4	75.0	0	5	60.0	0
Mammals*	9	100.0	0	17	64.7	5.9
Total	17	88.2	0	29	55.2	3.5

For assay of λ phage, faecal samples were plated onto MacConkey plates and incubated overnight at 37 °C. These samples were then replica-plated onto plates (Luria + 1 mM MgSO₄ + 0.2% maltose plates) containing newly inoculated lawns of logarithmically growing D0-28. As a positive control, a plate of *E. coli* U-7 (λ ⁺) was processed in the same manner as a test sample. After overnight incubation at 37 °C, the phages were collected by suspending the plate contents in 4 ml cold ϕ 80 buffer. Cells were removed by pelleting in a clinical centrifuge. The resultant supernatants were diluted in ϕ 80 buffer and assayed for phage using D0-28 and D0-29 indicator strains in soft agar overlays on nalidixic acid 30 μ g ml⁻¹. For further testing, plaques were probed with microcapillary pipettes and resuspended in 0.5 ml ϕ 80 buffer. For M13 phage assay, several loops of faecal bacteria from each sample MacConkey plate were used to inoculate 5-ml log phase cultures of B0-11 in TYE broth. This strain was used to enrich the male-specific phages. A culture of B0-11 inoculated with M13 was the positive control. Cultures were shaken overnight at 37 °C. Supernatants were obtained by sedimenting cells in a clinical centrifuge. Indicator cells for titration were grown to late logarithmic phase, sedimented and resuspended in an equal volume of 0.1 mM NaCl and shaken for 20 min. Cells were then pelleted and resuspended in 0.1 mM NaCl. Dilutions of supernatants in TE (10 mM Tris-HCl, pH 8.0 1 mM EDTA) buffer were plated with F⁺ and F⁻ indicator cells in Trypticase medium soft agar overlays.

* Cattle, horses, cats, dogs, goats and pigs.

specific phages amongst the 77 phage isolates studied. Nine of the male-specific phages, 11.7% of the isolates, were considered to be single-stranded DNA phages by their 'turbid' plaque morphology and were inactivated by anti-AE₂ serum.

In a study of 500 strains of human *E. coli*, Jacob and Wollman¹³ found only three isolates which were indistinguish-

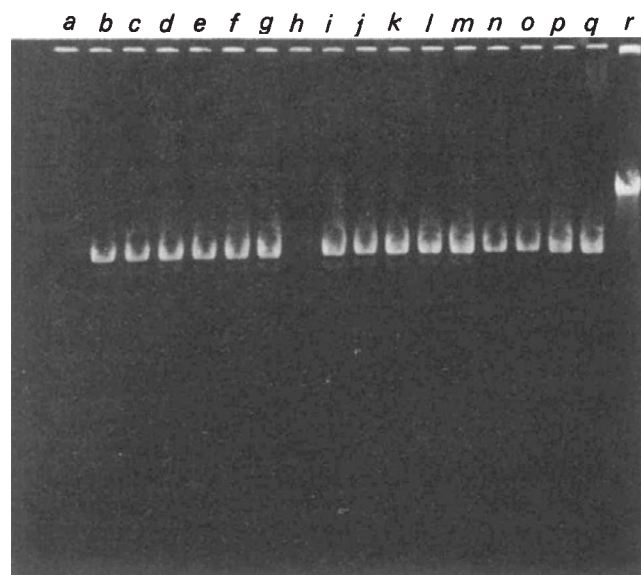


Fig. 1 Agarose gel (0.8%, 1.5 mm) electrophoresis in Tris-borate-EDTA buffer (pH 8.3) of supernatants from B0-11 cultures infected with plaque isolates. Aliquots of 30 µl of supernatants from phage-infected B0-11 cultures were added to 3 µl 2% SDS, 3 µl 0.25 M EDTA and 4 µl 0.1% BPB-50% glycerol and incubated at 65 °C for 10 min. Samples were then applied to the gel and analysed after electrophoresis for 3 h at 25 mA. Negative controls (a, h) were from two separate cultures of B0-11 cells alone. Positive controls (f, l and q) were native M13. Phage λ was applied to slot r. All other samples were phage isolates.

able from λ by immunity, host range and antiserum neutralisation. Fourteen other temperate phages of unique immunity types shared some, but not all, characteristics with λ. Our findings appear similar to those of Jacob and Wollman, published in 1956.

Our results can be used in assessing the potential for interaction of λ or M13 cloning vectors with wild-type phages in man's environment.

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Host range of *Agrobacterium tumefaciens* is determined by the Ti plasmid

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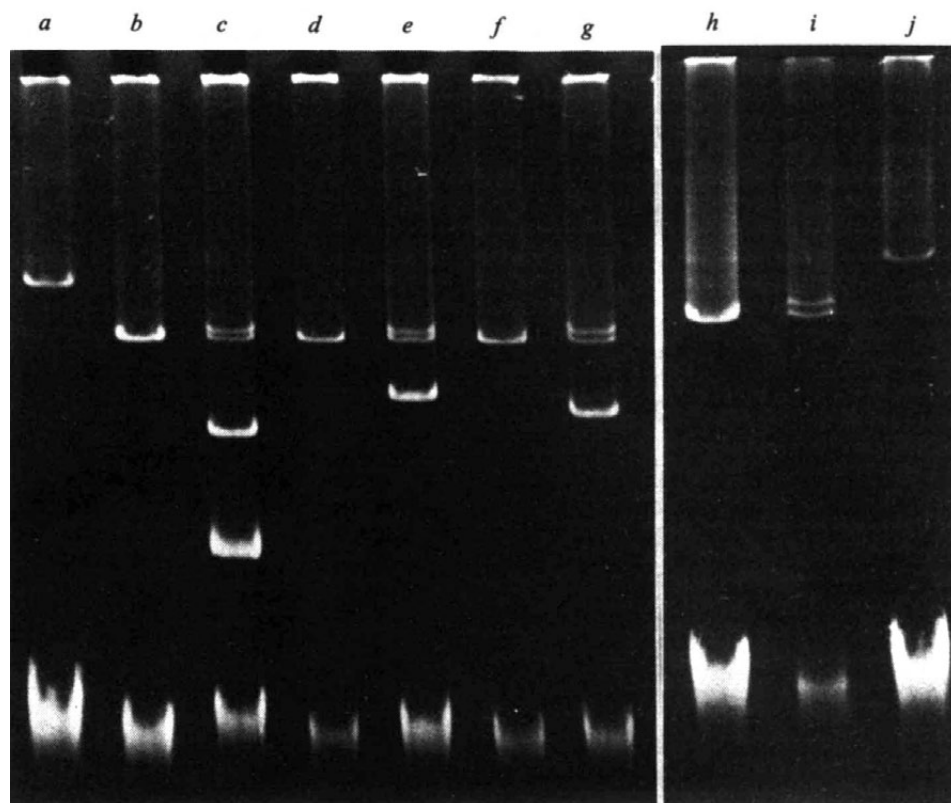
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The plant pathogen *Agrobacterium tumefaciens* can induce crown gall tumours in a wide range of dicotyledonous plants. To be virulent an *Agrobacterium* strain must contain a tumour-inducing (Ti) plasmid^{1,2}. Most Ti plasmids fall into one of two groups, based on whether they code for enzymes for octopine or nopaline utilisation^{3,4}. Tumours induced by strains carrying octopine or nopaline type Ti plasmids generally synthesise octopine or nopaline respectively^{3,4}. Normal plant tissues do not synthesise these unusual amino acid derivatives^{5,6}. Chilton *et al.*⁷ have analysed total DNA isolated from a cloned sterile tobacco crown gall callus and found sequences (T-DNA) complementary to ~5% of the Ti plasmid that are not found in normal tobacco callus. Thus, *Agrobacterium* can induce and maintain tumours by introducing genetic information stably into plant cells. It is evident that plasmid sequences have a central role in *Agrobacterium* virulence. Based on metabolic and physiological properties, most isolates of *A. tumefaciens* can be classified as either biotype 1 or biotype 2 organisms^{8,9}. They have a broad host range, inducing tumours on most dicotyledonous plants. Recently, however, strains isolated in Greece and Russia from grapevine tumours have been shown to have a limited host range^{10,11}. These organisms are also unique in that they do not fit into either of the previously described biotypes and have therefore been assigned to a new class, biotype 3 (refs 11, 12). We felt that these limited host range organisms could provide a system to study determinants of host range: if host range is determined solely by plasmid genes, the transfer of the Ti plasmid from a limited host range biotype 3 organism (assuming the biotype 3 organism had a Ti plasmid) to a biotype 1 organism that had been cured of its Ti plasmid, should yield a transformant of limited host range. The transformant should have a wide host range if this is a chromosomally borne trait. In this report we present data suggesting that the Ti plasmid has a major role in determining host range.

A. tumefaciens strains Ag57 and Ag63 isolated in Greece, and Ag158 and Ag162 isolated in Russia are biotype 3 organisms with a limited host range. Plasmids from these strains were isolated and subjected to electrophoresis through an agarose gel (Fig. 1). All of the isolates contained two large plasmids with molecular weights (MWs) of ~140 × 10⁶ and 130 × 10⁶. Both Ag63 and Ag158 contained an additional plasmid with MWs of ~65 × 10⁶ and 72 × 10⁶, respectively. Strain Ag162 contained two additional plasmids with MWs of about 55 × 10⁶ and 28 × 10⁶. Plasmids from the four strains were isolated and used to transform strain A136 as previously described¹³. This latter strain is a biotype 1 organism that has been cured of its virulence plasmid pTiC58 but retains a cryptic plasmid pATC58 (ref. 14, see Fig. 1). Transformants were selected on a minimal medium with octopine added (400 µg ml⁻¹) as the sole source of carbon and nitrogen. All four donor strains can catabolise octopine (M.F.T. and E.W.N., unpublished results; C.G.P., unpublished results) and we hoped that, as with biotype 1 organisms, this characteristic would be a

Fig. 1 Plasmids from *A. tumefaciens* strains with a limited host range and their respective transformants. Plasmid was isolated by a modified version of the Currier-Nester technique²⁴ as described by Casse *et al.*¹⁴. Samples were subjected to electrophoresis through a 0.7% agarose gel as previously described⁷ except that a vertical gel apparatus was used. Plasmid bands were visualised by staining with ethidium bromide (1 µg ml⁻¹) and illuminating with a Blak-Ray Transilluminator (UV Products). Samples are as follows: *a* and *j*, A136; *b*, Ag162Tr; *c*, Ag162; *d*, Ag158Tr; *e*, Ag158; *f*, Ag63Tr; *g*, Ag63; *h*, Ag57Tr; *i*, Ag57.



plasmid-borne trait. This proved to be true as octopine-utilising transformants were recovered. They were typical biotype 1 organisms in that they were 3-ketolactose positive and grew on the selective medium of Schroth *et al.*¹⁵. In each case the transformants (Tr) contained the 130×10^6 MW plasmid found in the parental strain (Fig. 1). When plasmids from the parents and transformants were digested with the restriction endonuclease *Sma*I and the products separated by electrophoresis on an agarose gel, restriction fragments of the plasmids from the transformants formed a subset of the restriction bands found in their respective parents (data not shown).

The *Bst*I restriction patterns of the plasmids from the transformants are presented in Fig. 2. The pattern of the pATC58 plasmid is not visible because its relative yield in the plasmid preparations is low (see Fig. 1). The patterns of the transformants are strikingly similar, suggesting that the plasmids are closely related. The banding patterns of the plasmids contained in Ag158Tr and Ag162Tr actually appear identical although their respective parents are obviously different (Fig. 1). Their pattern differs by only one band from that obtained with the plasmid from Ag63Tr. However, the restriction patterns of the plasmids in the transformants bear little resemblance to that of the pTi-B6-806 plasmid, the octopine Ti plasmid present in the biotype 1 strain A277. This is of interest because the restriction patterns of all octopine type Ti plasmids investigated to date have been virtually identical¹⁶. This is exemplified by the *Bst*I digestion patterns of two typical octopine plasmids, pTi-A6 and pTi-15955 (Fig. 2). These data suggest that octopine plasmids present in limited host range strains form a family of plasmids distinct from the wide host range octopine plasmids.

The host range of strains Ag57, Ag63, Ag158, Ag162 and their transformants is presented in Table 1. The virulence response of the transformant was virtually identical to the donor strain with all host plants tested. Significantly, when the donor strain gave an attenuated virulence response, as seen with Ag57 and Ag63 on sunflower and *Datura*, the transformants gave the same response. With tobacco, a slight difference in virulence between the limited host range donor strains and their transformants is suggested. Both Ag57 and Ag63 formed small,

Table 1 Virulence of *Agrobacterium* strains on various host plants

Bacterial strain*	Host plants						
	Grapevine <i>Vitis vinifera</i> c.v. Sultanina	Tomato, c.v. San Pietro	<i>Kalanchoë</i> <i>diagrammontiana</i>	<i>Datura</i>	Sunflower	Tobacco (<i>Nicotiana tabacum</i> c.v. Turkish)	Tobacco (<i>N. glutinosa</i>)
Ag57	+	-	-	-/(+)	-/(+)	(+)	ND
Ag57Tr	+	-	-	-/(+)	-/(+)	-	ND
Ag63	+	-	-	-/(+)	-/(+)	(+)	ND
Ag63Tr	+	-	-	-/(+)	-/(+)	- ⁺	ND
Ag158	+	-	ND	-	-	-	+
Ag158Tr	+	-	ND	-	ND	-	+
Ag162	+	-	ND	-	ND	-	+
Ag162Tr	+	-	ND	-	ND	-	+
A277	-	+	+	+	+	+	+
A136	-	-	-	-	-	-	-

The symbols used to describe the virulence response are; +, large active galls; (+), small galls that appear late compared with A277; -/(+), small galls that appear late and not at every inoculation site; -, no gall formation; ND, not determined.

* Strains Ag57, Ag63, Ag158, Ag162 and their transformants (Tr) are described in the text. The derivation of strains A136 and A277 have been described previously^{2,16}.

† This strain was avirulent on *Nicotiana tabacum* c.v. Turkish but gave very small, late appearing galls on *Nicotiana tabacum* c.v. xanthi.

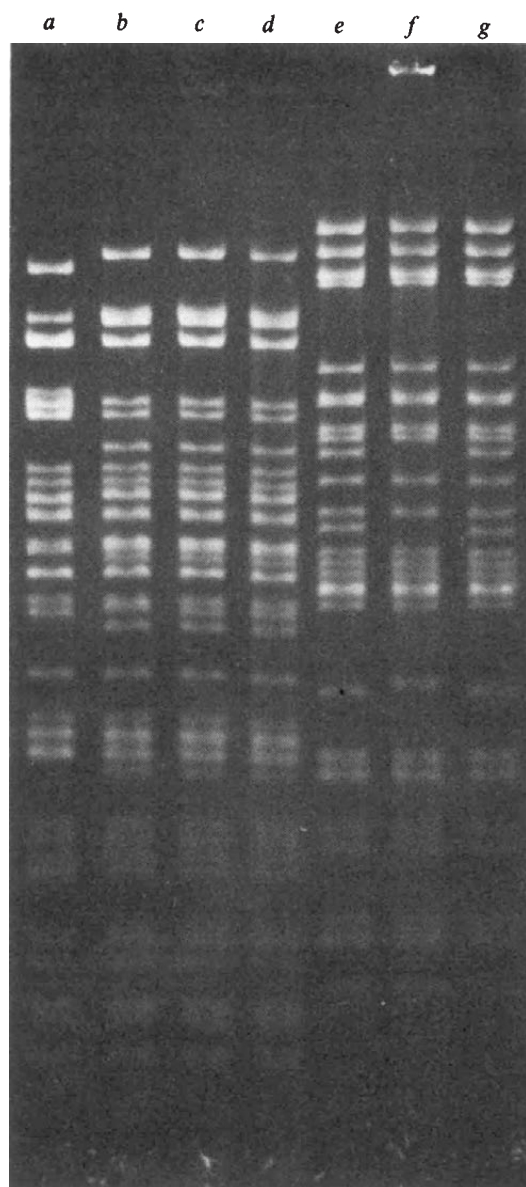


Fig. 2 *Bst*I cleavage patterns of isolated plasmids. Plasmid (0.5 µg) isolated by the Currier-Nester procedure²⁴ was digested with *Bst*I at 37 °C in a buffer consisting of 100 mM Tris-HCl (pH 7.6), 6 mM MgCl₂ and 100 mM NaCl. The products were separated by electrophoresis through a 0.7% agarose gel as previously described⁷. The restriction bands were visualised as in Fig. 1. Plasmid was isolated from the following strains: a, Ag57Tr; b, Ag63Tr; c, Ag158Tr; d, Ag162Tr; e, A277; f, A6; g, 15955.

slowly developing galls on *Nicotiana tabacum* c.v. Turkish, while the transformants formed no galls at all. Ag63Tr did form very small galls on *Nicotiana tabacum* c.v. xanthi. The data also show that although A277 has a wide host range, it is not virulent on grapevine. This inability to induce galls on grapevine must be a function of the Ti plasmid and not the chromosomal DNA as all the limited host range transformants formed large active galls on grapevine. It is clear from these data that the major determinant of host range for the strains tested is plasmid coded. The results obtained with tobacco suggest that chromosomal DNA, or possibly the other plasmids harboured by Ag57 and Ag63 might modulate host range to a limited extent.

How plasmid genes determine host range is not known. Apparently, effective binding of agrobacteria to plant cells is a prerequisite to tumour formation^{17,18}. *A. tumefaciens* strains

containing Ti plasmids bind more effectively to tobacco and carrot cells than do strains that do not harbour Ti plasmids^{19,20}. These data suggest that it is the Ti plasmid that enables *Agrobacterium* to bind to plant cells. It is possible that the Ti plasmids from the limited and wide host range isolates allow *Agrobacterium* to bind to cells of different plant species. An analogous system for enteropathogenic *Escherichia coli* has been shown to exist. Plasmid sequences code for surface antigens that allow the bacteria to bind to animal cells; different antigens allow binding to cells from different animal species²¹. Without binding, *E. coli* strains that are able to produce enterotoxin are not pathogenic²².

An additional ancillary conclusion that may be drawn from these data is that all octopine Ti plasmids do not form a homogeneous group as previously thought¹⁶. Current studies in our laboratories are aimed at determining the extent to which these and other limited host range octopine Ti plasmids differ from their wide host range counterparts.

Since submission of this letter we have transferred a wide host range Ti plasmid into strain Ag63. The transconjugant assumes the wide host range of the donor strain (V. Knauf and A. Montoya, unpublished observations). Our data on the importance of the plasmid in determining host range agree with the conclusions of Loper and Kado²³.

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Erratum

In the News Feature 'Recombinant DNA: have recent experiments assessed all the risks?', *Nature* **282**, 773, 1979, column 3 lines 5–7 on page 773 should read 'intact recombinant λ or plasmid DNAs with single polyoma inserts gave variously 0, 6 and 9% tumorigenicity'. (Not 0.6 and 9%.)

BOOK REVIEWS

Other men's minds

Kevin Connolly

"PEOPLE come and go, but the creative sources of great historical events and the important ideas and deeds remain." This sentiment, expressed in the penultimate sentence of Alexander Romanovich Luria's short autobiography gives the key to the book. It is one of that small genre of intellectual autobiographies; it is fascinating because of the man and his work, and important because it spans almost the whole of Soviet psychology as it emerged from the deep and frowsty conservatism of tsarist Russia after the October revolution.

Luria was born in Kazan in 1902, into the Russian intelligentsia class. Arguably he was the best known Russian psychologist of his day, a prolific writer and an inveterate traveller. The cataclysmic changes of 1917 had a profound effect upon him: "The revolution freed us, especially the younger generation, to discuss new ideas, new philosophies and social systems." Although not aware of the real causes of the revolution Luria, in company with other young people, threw himself into the new movement because of the opportunities which it presented. In the opening chapter the liberation offered by the revolution in those early years comes across as a passionate, almost spiritual, excitement which served to fuel his intellectual endeavours.

Amidst the turmoil in Kazan University in the immediately post-revolutionary years Luria became absorbed with the then rather formless set of topics in the social and biological sciences which we now think of as psychology. After graduating in 1921 he began to investigate the effects of physical work on mental activity. His early studies on the role of verbal instructions on reaction time led to an enduring interest in the regulatory role of speech. He was also much interested at this time in psychoanalysis and had founded the Kazan Psychoanalytic Association.

In 1923 he moved to the Institute of Psychology in Moscow. The new Director, Kornilov, was engaged in eliminating all traces of subjective psychology and replacing it with objective methods — his "reactology". While appreciating the need for objective methods Luria avoided the sterility of this simple mechanistic scheme, his interest in psychoanalysis probably bolstered him in this. He designed a technique which he called the "combined motor method" to study the influence of

The Making of Mind: A Personal Account of Soviet Psychology. By A.R. Luria. Edited by Michael Cole and Sheila Cole. Pp.234. (Harvard University Press: Cambridge, Massachusetts, and London, 1979.) \$15; £9.

emotions on voluntary behaviours. This early work, which was never published in Russian, shows clearly one of his trademarks — the application of objective methods to real life psychological problems.

In 1924 he met L.S. Vygotsky whom he describes quite simply as a genius. For ten years he worked closely with Vygotsky whose ideas plainly had a profound and enduring effect upon him. Vygotsky's 'cultural psychology' owed much to Marx's methods of analysis and to the intellectual climate released in Russia after the revolution. Inspired by Vygotsky's ideas Luria, Vygotsky and a small group of students set themselves the task of reconstructing psychology with man in his proper historical and cultural context. These ideas led Luria in the early 1930s to make two expeditions to Uzbekistan in Central Asia. Here a traditional peasant society profoundly influenced by a conservative Islamic culture was in the process of being transformed by collectivisation, literacy campaigns and formal education. The purposes and methods, along with an outline of the findings obtained from the two expeditions are described. This brief account of the work on cultural differences in thinking done almost 50 years ago is one of the most interesting chapters in an absorbing book. It deals with work which is little known in the Soviet Union and even less in the west. Following these pioneering studies Luria turned to work on twins in an effort to disentangle nature and nurture as they shaped psychological processes. From the account provided one cannot help but be struck by the originality displayed in the work and by the way in which Vygotsky's theoretical framework is woven throughout it.

Luria was perhaps best known as one of the founding fathers of neuropsychology. In the late 1930s he completed his medical training in Moscow and set about becoming, as he puts it, "a practical neurologist". He made notable contributions to our understanding of the aphasias, once again informed by

Vygotsky's theory. When Russia entered the second world war in 1941 he was commissioned to organise a hospital for patients suffering brain injuries. The development of his ideas and methods of rehabilitation, especially in relation to speech, are simply and vividly described. The link between the war work and subsequent studies on the relationship between brain and behaviour, especially in connection with language function, is explored.

The final chapter in Luria's little memoir has the beguiling title, "Romantic Science". He distinguishes between classical science which is essentially reductive in nature, and romantic science which is concerned with the broader canvas of the whole person. In psychology the distinction and the corresponding dilemma have often been formulated in terms of the nomothetic versus idiographic approaches. Luria's work takes from both of these but in a period when the nomothetic approach is in the ascendancy in western psychology his clear statement about the sources of ideas and about the importance of preserving the manifold richness of the subject, which he illustrates by his detailed studies of a man with an amazing memory and another man whose mind was shattered by a severe brain injury, provides a timely caution to us all.

In their introduction the editors provide a valuable historical context to Luria's psychological work. The final chapter is a sensitive and insightful essay by the editors in which they seek to give a picture of Luria the man. This is plainly difficult but the vicissitudes of his life are outlined and provide some explanation for the striking changes in direction which his work took, changes often brought about by political necessity. The two great influences on the development of Luria's psychological work were the revolution, which opened it, and Vygotsky whose ideas provided a connecting framework which ran throughout it. The atmosphere of the intellectual life which is revealed shows Luria to be a man possessed of boundless energy and insatiable curiosity — a great man. The book is quite simply a gem. Because of its simplicity and directness it reveals that most fascinating of things, another man's mind at work. □

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Objects of the Universe

David W. Hughes

Catalogue of the Universe. By Paul Murdin and David Allen, with photographs by David Malin. Pp.256. (Cambridge University Press: Cambridge, 1979.) £9.50.

cătălögule, *n.* a list or enumeration systematically arranged in alphabetical or other order, often with the addition of brief particulars.

ūn'īverse, *n.* The whole of created or existing things regarded collectively; all things, including the earth, the heavens, and all that is in them, considered as constituting a systematic whole.

ON reading these two dictionary entries the enormity of the task the authors had set themselves seemed overwhelming. Murdin, Allen and Malin have, however, assembled a much simplified catalogue of astronomical objects and planetary features. The book is a picture gallery of galaxies, stars, nebulae and planets, each photograph being accompanied, on average, by an equal area of prose. The balance is reasonable too: 26% galaxies, 36% stars and nebulae, and 38% planets.

In the preface the authors state that the photographs are the core of the book, and a "best-buy" policy has been adopted in

selecting the ones for inclusion. They then bravely go on to say: "We hope that an astronomer browsing in the sections which lie outside his special field thinks that the best-buy choices are justified, even if he happens to disagree with us in the area he knows about." Well I did just that and obviously this makes me very worried. My field is the Solar System, especially the minor bodies in it — comets, asteroids, meteorites and meteoroids. The photographs in this section were poor — I'm sure I could have selected much better ones myself. Also having NASA slides in my collection of many of the Moon, Mercury and Mars photographs used in the book, underlined the fact that many of the reproductions in these sections are not of the best. However, as I wandered away from my field and browsed in other pastures I became more and more impressed. The Lagoon nebula looked sharper than I had ever seen it before, thanks to some of the special photographic techniques used for the illustrations. On the other hand, even here a common illustration like the Lund Observatory's composite photograph of the Milky Way has been so badly reproduced that much of the detail you know exists on the original is invisible. But I'm still worried about the author's admonition. May be if I meet an expert of that speciality in some distant pasture, wouldn't he be as disillusioned with his area as I was with mine? It's quite surprising isn't it how impressive certain books can be when they discuss things you

haven't encountered before, only to become painfully weak when they turn to your own speciality.

The text also worries me. Common catalogues like stamp catalogues and horticultural catalogues at least tell you how big the stamps are, their perforation, the issue dates and print numbers; and when reading about the plants I expect to learn how tall and hardy they are or whether they like shade or sun. Common catalogues abound with quantitative details. Not so this one. Time and again I get the impression the reader is being talked down to. I want to know how rare, hot, massive, dense, luminous, old (etcetera, etcetera) these objects are, I'm not told often enough. If I've gone to the trouble of looking up "infrared radiation" in the glossary I want to be told more than that it is just heatwaves. Any author who writes that fireball trains are nearly as wide as the Full Moon sows seeds of doubt in my mind about his other prognostications.

The underlying, motivating, idea behind the book is extremely good. A catalogue of the objects of the Universe, well illustrated with a concise factual text would be most useful. Unfortunately with this book both the illustrations and the text let it down. Remove these and there is little to say other than that it has a pretty cover. □

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Physical optics

P.W. Hawkes

Optical Image Formation and Processing. By M. Françon. Pp. 213. (Academic: New York, San Francisco and London, 1979.) \$19.50; £12.80.

THE past twenty years have seen a number of major developments in optics, but for the newcomer, their relative novelty simply means that older textbooks will not cover them and that some of the newer ones, or new editions of the old, will treat them as difficult advanced topics or in brief, all-too-often obscure appendices. Professor Françon is too experienced a teacher of optics to fall into either of these traps. He has written a short introductory text on physical optics, in which such topics as partial coherence, transfer functions, holography, speckle interferometry, image processing, lasers and optical waveguides

are treated alongside and in exactly the same tone of voice as, for example, Babinet's principle and the Michelson interferometer. Students encountering physical optics for the first time will find the style clear and the mathematics simple and easy to follow. Only in one case did I find the clarity deceptive, namely, the treatment of speckle; here, I feel that the newcomer will find himself following the discussion effortlessly but without the least idea of what speckle is all about.

In a book intended for beginners, the quality of the translation is particularly important. The translator, B.M. Jaffé, has produced a text that is smooth and lucid for the most part, though here and there a technical phrase has eluded him. The only one that matters is "pattern recognition", for which B.M. Jaffé writes "form recognition" (from *reconnaissance des formes*). From personal experience, I know how easy it is to be hypnotised into translating into perfectly grammatical but somewhat unnatural English and B.M. Jaffé is occasionally, though not often, guilty of this: "If one agrees to allow the

frequency to vary . . ." (p 59), for example, and " . . . it will be necessary to remind the reader of the basic properties . . ." (p 98). Here and there, I felt he might have inserted (if the author agreed) a helpful phrase; thus again on p 98 it would have been useful to mention that fig. 8.1 is commonly known as the Hurter-Driffield or H-D curve and to put "log W" in the text. On p 190, he really ought to have noticed that, although "The first of the two relations (A.28) is often given the name of Parseval's theorem", this is in fact wrong and Parseval's theorem states something different, which is easily derived from the relation in question.

Nevertheless, these are only very minor blemishes in a book that first or second-year undergraduates, revising for exams, will find immensely helpful particularly if Academic Press produce a paperback that they can afford.

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Semiconductor physics

Andrew K. Jonscher

Semiconductor Physics. By P. S. Kireev. Pp. 693 (MIR Publishers/Central Books: London, 1977.) £5.95.

THIS is an interesting novelty — a translation from Russian into English made and published in the USSR and offered at the bargain price, by any standards, of less than six pounds for nearly 700 pages in hardback. The first point to make is that the translation is remarkably good coming from a non-native English speaker and it would be churlish to quibble over small points of odd usage of English. That aspect is quite satisfactory.

The material is organised into the following chapters (with numbers of pages): introduction (31), band theory (138), electron and hole statistics (54), kinetic phenomena (135), scattering theory (92), recombination (36), contact phenomena including p-n junctions (35), optical and photo-electric phenomena (101), Appendix — group theory (61). The size of the chapters reflects fairly closely the Author's predilections and the strong points of his expertise, which evidently centre on theory; all treatment of 'concrete devices' is excluded. The quantum mechanical treatment of the band theory of solids is very thorough — certainly at postgraduate level — and includes the effects of magnetic fields, surfaces and point defects, but not strongly disordered solids. Simple examples of band structure calculations are also included. Carrier statistics is treated conventionally, includes a lot of detail and covers, in particular the quantisation in high magnetic fields.

The treatment of transport begins with Boltzmann's transport equation — going into considerable detail with tensor effective masses — and includes the treatment of galvanomagnetic phenomena, especially the Hall effect, heat conductivity and thermoelectric effects. It is noteworthy that frequent examples are given of experimental data to illustrate particular developments in the theory. The principal carrier scattering mechanisms are described in considerable details, and a rather unusual feature of the book is that lattice vibrations and specific heats are dealt with as a sub-section of this chapter. There is a brief treatment of high-field effects and deviation from Ohm's law, but this general subject is treated in a rather disappointingly sketchy manner and manages to give a confusing picture of the Poole, Stark, Poole-Frenkel and Zener effects. The important topic of transferred

electron phenomena receives barely one page and one not very informative diagram: — one would have expected more after the very thorough preparation in earlier chapters. There is nothing about effective carrier temperatures in high fields and band-to-band avalanche multiplication, and saturated drift velocity is shown in a diagram but is not discussed in any detail.

After the very detailed treatment of the band theory, the presentation of the physical aspects of carrier recombination is decidedly perfunctory and the subject of minority carrier injection does not receive any significant amount of attention, either at the level of analysis or of a physical description of ambipolar flow and preservation of neutrality. The chapter devoted to contacts includes a very sketchy description of the p-n junction but the voltage-current characteristics of neither of these are derived. This is perhaps taking the avowed aim of excluding devices a little far, as the I-V characteristic may be considered to be both fundamental to the theory of semiconductors and highly illuminating physically of a range of transport phenomena.

By contrast with the above, optical and photoelectric phenomena are treated again quite thoroughly, especially free carrier absorption, cyclotron resonance, plasma reflection, intrinsic absorption in considerable detail, lattice absorption and impurity absorption. Likewise, photoconductivity, photovoltaic effects and photoelectromagnetic effects are given reasonably good coverage, with illustrations relating to experimental results.

There is an appendix on group theory which is useful for the beginner, although the precise logic of including this rather than any other supporting material is not obvious. A good feature of the book are the brief summaries at the end of each section which should be useful for revision and for rapid scan of the principal features.

Although carefully selected passages of this text would be suitable for an undergraduate course, the treatment is definitely postgraduate, and rather specialised at that. The book is literally packed solid with information — the typographical layout is very condensed, with 46 lines to a page of slightly smaller format than an equivalent Western text with 38 — 39 lines. Similarly, mathematical expressions, of which there is a great deal, are tightly packed in the text and most of the diagrams are very small by our standards. Thus, although the reading process may be more tiring, the book contains a good 20% more information per page than its equivalent here.

The quality of paper is not high — especially as the book is liable to get thumbled readily in extensive usage. A very serious defect — not untypical of Soviet books — is the omission of an index, especially in a text that is more likely to

serve as a reference source rather than as a regular reading text.

Altogether, a very mixed impression. A first-class bargain in terms of the sheer quantity of information per pound sterling, good coverage of theoretical aspects such as band theory, transport and optical phenomena, but quality of treatment falling off rapidly in other areas. In those areas in which it is strong it should form a good reference source, although I would dread having either to teach or to read a course of that level of detail. Perhaps the publishers may be able gradually to take into account the different standards of presentation normally expected in the West, thus further increasing the attractiveness of the product, even if that meant raising the price by as much as 50% — it would still remain a bargain! □

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Yeast identification

B.H. Kirsop

A Guide to Identifying and Classifying Yeasts. By J.A. Barnett, R.W. Payne and D. Yarrow. Pp. 315. (Cambridge University Press: Cambridge, 1979.) £32.50.

THE yeasts are a heterogeneous and important group of organisms which are widely distributed, commonly isolated and often in need of identification. This *Guide* has been written to help overcome difficulties in identification and is intended to be used in conjunction with the standard work (*The Yeasts*, edited by Lodder, North Holland, 1970). Those experienced in yeast identification will meet little difficulty and so the present work must be judged by the extent to which it helps the inexperienced.

The *Guide* provides condensed but useful descriptions of methodology and of the principles of classification. It lists the salient properties of each genus and provides a particularly valuable tabulation of the major physiological and morphological attributes characteristic of each species. Identification is based on keys, a general one being provided for yeasts as a whole; there are also special keys to smaller groups, such as those likely to occur in particular habitats or those possessing specific morphological or physiological attributes. Testing with unnamed yeast showed that the keys are easy to use and readily yield names. In a

significant proportion of cases this name differed from that deemed appropriate when all the properties of the organism were considered; the discrepancies arose either because an organism was atypical for its group in a characteristic used in the key — all microbiologists know well the atypical organism — or because of difficulty in interpreting test results. The authors recognise the problem, for they provide a useful summary of the features which distinguish each species from all, or

all but a named few, other species; and this helps the inexperienced to detect errors.

In spite of this drawback, the *Guide* provides much useful information which is particularly valuable for the expert, for it brings together in one place much information published since the 1970 edition of *The Yeasts*. Since then many new species have been described and massive reorganisations of taxonomy have been recommended, accepted by the authors and incorporated into the keys. Although

this makes it difficult to use the *Guide* in conjunction with the 1970 edition, as recommended by the authors, the ready accessibility of this information is nevertheless most welcome. One is tempted to wish that the authors had produced a still more substantial monograph which would have been of great value to the beginner and to the expert alike. □

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Dealing with information in the nervous system

H. B. Barlow

Information Transmission in the Nervous System. By A. M. Uttley. Pp. 111. (Academic: London and New York, 1979.) £7.50; \$15.50.

It is obvious that Pavlov's dogs, who salivated when a bell sounded, had in some way formed an assessment of the conditional probability of food, given the sound of a bell; because the dogs' past history had been controlled so that this probability was high, they salivated on the sound of a bell, without waiting for the food. In this sense, the nervous system must be able to compute conditional probabilities, and this is the starting point for the work, stretching back over 30 years, that Albert Uttley summarises in this book.

I think he is right in giving central importance to the question how conditional probabilities are assessed in the nervous system, and I also like another feature of his approach. Given its central importance, he says, let us explore the hypothesis that modifiable synapses in the nervous system are commonly arranged in such a way that the modified synapse stores a conditional probability. The book is a development of this theme, and centres on the "informon", an element for which a stored variable, Y_i , representing the effectiveness of a synapse, is given by

$Y_i = -k \log(P(X_i \text{ and } Y) / (P(X_i) \times P(Y)))$ where k is a positive constant, and X_i and Y are two binary signals, the X 's usually representing inputs and Y an output. Y_i is thus the negative of Shannon's Mutual Information Function for X_i and Y . Before going into further details two warning are required.

First, the title might mislead the unwary reader. It is not a general treatise on how information, in the general sense, is

transmitted in the nervous system. Rather, the title was chosen, I suspect, to emphasise that "informons" adjust themselves in accordance with "information" in the specialised Shannon sense, rather than just messages; they store the conditional probabilities that enable them to deal effectively with information.

The second warning is that, although I am sympathetic to the author's general purpose and train of thought, I did not find the book easy to read and follow. The author has perhaps lived with the ideas so long that he no longer has the perspective necessary in order to explain them effectively to newcomers.

The book contains eleven short chapters. The first four explain the concepts and illustrate how a theoretical "informon" might be realised by a nerve cell with modifiable and unmodifiable synaptic inputs. The next two chapters match the conceptual informon's behaviour against that of granule cells in the hippocampus, as shown by the work of Bliss and Lomo in rabbit and Douglas and Goddard in rat. He then deals with classical conditioning, the use of informons for pattern recognition, the interpretation of plasticity in the visual cortex, and the extension of the ideas to operant conditioning.

The book is a valiant and rather isolated effort in an original direction. It should be read by those who seek an explanation for quite complex psychological facts in elementary properties of synapses and nerve cells, and it thus forms a contrast with explanations for those same facts in terms of "programs" that could be realised by any kind of computing machinery. Conditional probabilities and Shannon's Information are quantities that form conceptual bridges between the two approaches, and the most valuable feature of Uttley's work may be to show how much can be achieved directly by neural hardware adapted to computing these quantities. □

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Objectives of agricultural policy

R. F. Lord

Government and Agriculture: A Spatial Perspective. By I. Bowler. Pp. 127. (Longman: Harlow, 1979.) Paperback £4.50.

DR BOWLER is one of a growing band of geographers concerned not only to describe, but also to hypothesise. In this book he takes the view that "a spatial perspective can provide additional insights into the government — agriculture relationship". That he only partially succeeds in his task is due largely to the fact that objectives of agricultural policy rarely contain a specifically spatial dimension and that even where they do, statistically acceptable data are seldom available on a spatial basis in order to measure achievement.

The first part of the book drawing on experience in a number of developed countries, with a wealth (surfeit?) of references, considers how agricultural policy has been influenced by the changing nature of the "agricultural problem" and concludes that for the most part there has been a failure to achieve the policy goals so optimistically set by governments.

The second part of the book relates solely to the development of UK agricultural policy in the post-war period up to 1973. Only in the last two chapters (6 and 7) is attention devoted to the spatial aspects of the problem (which considering the title of the book is surprising). Generally the ineffectiveness of measures in a spatial sense is demonstrated. However, Dr Bowler's concern for the spatial dimension is justified in his concluding paragraph where he refers to the need for more research of this type if problems of formulating adequate regional policies in the EEC are to be overcome. □

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